Subtitle B.—*Regulations of the Offices of the Department of Education*—Continued

Old part number and title—45 CFR Part	New part number—34 CFR Part
197 Teacher centers program	240
198 Training for higher education personnel	241
186 Indian Education Act—General provisions	250
186a Entitlement grants—Local educational agencies and tribal schools	251
186b Indian-controlled schools—Establishment	252
186c Indian-controlled schools—Enrichment projects	253
186d Demonstration projects—Local educational agencies	254
186e Educational services for Indian children	255
186f Planning, pilot, and demonstration projects for Indian children	256
186g Educational personnel development	257
186h Educational services for Indian adults	258
186i Planning, pilot, and demonstration projects for indian adults	259
186j Adult education research and development projects	260
186k Adult education surveys	261
186l Adult education dissemination and evaluation projects	262
187 Indian fellowship program	263
180 Desegregation of public education	270
185 Emergency school aid	280
161 Career education, State allotment program	295
161a Career education, discretionary programs	296
Chapter III—Office of Special Education and Rehabilitative Services (Part 300–399)	
121a Assistance to States for education of handicapped children	300
121m Incentive grants	301
116b State operated programs for handicapped children	302
121b Regional resource centers	305
121c Centers and services for deaf-blind children	307
121d Early education for handicapped children	309
121e Auxiliary activities	315
121f Training personnel for the education of the handicapped	318
121g Recruitment of personnel and dissemination of information	320
121h Research in education of the handicapped	324
121k Regional education programs for handicapped persons	338
121o Captioned films loan service for the deaf program	330
121p Educational media loan service for the handicapped program	331
121q Educational media research, production, distribution, and training	332
121r Center for educational media and materials for the handicapped program	333
195 Gifted and talented children's education program: General	345
195a Gifted and talented children's education: State-administered program	346
195b Gifted and talented children's education program: Discretionary grant program	347
1361 The State vocational rehabilitation program	361
1362 Project grants and other assistance in vocational rehabilitation	362
1369 Vending facility program for the blind on Federal and other property	369
1370 Evaluation standards	370
Chapter IV—Office of Vocational and Adult Education (Part 400–499)	
104 State vocational education programs	400
105.1–105.507 (Appendices A, B) Commissioner's discretionary programs of vocational education	408
161f Youth employment program	419
163 Adult education State programs—General provisions	425
166a State administered adult education programs	426
166b National adult education development and dissemination program and planning grants	431
166c Adult education programs for immigrants and Indochina refugees	432
163 Community schools and comprehensive community education program	440
163a Community education—State program	441
163b Community education—Grants to local educational agencies	442
163c Community education—Grants to public agencies and nonprofit private corporations	443
163d Community education—Training grants to institutions of higher education	444
Chapter V—Office of Bilingual Education and Minority Languages Affairs (Parts 500–599)	
123 Bilingual education: General Provisions	500
123a Bilingual education: Basic projects	501
123b Bilingual education: Demonstration projects	502
123c Bilingual education: State educational agency projects for coordinating technical assistance	503
123d Bilingual education: Support services projects	504
123i Bilingual education: Materials development projects	505
123e Bilingual education: Training projects	510
123f Bilingual education: School of education projects	514
123h Bilingual education: Fellowship program	515
123g Bilingual education: Desegregation support program	520
105.601–105.607 Bilingual vocational training program	525
105.611–105.617 Bilingual vocational instructor training program	526
105.621–105.127 Bilingual vocational instructional materials, methods, and techniques program	527
122a Indochina refugee children assistance	537
Chapter VI—Office of Postsecondary Education (Parts 600–699)	
149 Commissioner's recognition procedures for national accrediting bodies and State agencies	603
199a State postsecondary education commissions program—intrastate planning	605
137 Educational information centers program	606
173 Community service and continuing education programs	610
279* College housing programs administered by the Department of Education	614
170 Financial assistance for construction, reconstruction, or renovation of higher education facilities	617
101 Grants to land grant colleges and universities	621
169 Strengthening developing institutions program	624
189 Veterans' cost-of-instruction payments to institutions of higher education	629
182 Cooperative education programs	631
150 Law school clinical experience program	639
159 Talent search program	643
154 Educational opportunity centers	644
155 Upward bound program	645
157 Special services for disadvantaged students program	646
161l Biomedical sciences program	647
179 Graduate and professional study fellowships and institutional grants	648
194 Public service education program	649
196 Domestic mining and mineral and mineral fuel conservation fellowships	650
146 Modern foreign language and area studies	655
148 Higher education programs in modern foreign language training and area studies	662
146a Citizen education for cultural understanding program	667
168 General provisions relating to student assistance programs	668
174 National direct student loan program	674
175 College work-study and job location and development program	675
176 Supplemental educational opportunity grant program	676
177 Guaranteed student loan program	682
178 Student consumer information services	686
190 Basic educational opportunity grant program	690
192 State student incentive grant program	692
178a Incentive grants for State student financial assistance training program	695

Subtitle B.—*Regulations of the Offices of the Department of Education*—Continued

Old part number and title—45 CFR Part	New part number—34 CFR Part
Chapter VII—Office of Educational Research and Improvement (Parts 700–799)	
1400 General	700
1403 Eligibility and application for research grant assistance	701
1410 Miscellaneous requirements	702
1430 Experimental program for opportunities in advanced study and research in education	703
1440 Government in the Sunshine Act regulations	705
1450 Research grants program	708
1451 Basic skills research grants program	710
1470 Education and work grants program	714
1480 Program of research grants on organizational processes in education	716
1490 Education equity research grants programs	718
1495 Law and government studies in education	720
164 Capacity-building for statistical activities in State educational agencies	726
1501 Support for improvement of postsecondary education	730
182a National alcohol and drug abuse prevention program	740
160f Women's Educational Equity Act program	745
161b Metric education program	752
161c Arts education program	753
161e Consumers' education program	755
161g Law-related education	757
161h Financial assistance for environmental education projects	758
161m Population education program	763
162 Basic skills improvement and educational proficiency	765
162a National basic skills improvement	766
162b State basic skills improvement	767
162c Educational proficiency	768
184 Ethnic heritage studies program	769
130 Library services, public library construction, and interlibrary cooperation	770
131 College library resources program	773
134 Grants to State educational agencies for educational improvement, resources, and support	774
132 Grants for training in librarianship	776
133 Library research and demonstration	777
136 Strengthening research library resources	778
151 Territorial teacher training assistance program	790
172 Teacher corps	793
1460 State dissemination grants program	795
193 National diffusion network program	796

*24 CFR Part number.

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Friday
November 21, 1980

Part VI

Department of Health and Human Services

National Institutes of Health

Recombinant DNA Research, Actions Under Guidelines

DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Recombinant DNA Research; Actions Under Guidelines

AGENCY: National Institutes of Health, PHS, HHS.

ACTION: Notice of Actions under NIH Guidelines for Research Involving Recombinant DNA Molecules.

SUMMARY: This notice sets forth actions taken by the Director, NIH, under the January 1980 NIH Guidelines for Research Involving Recombinant DNA Molecules (45 FR 6724).

EFFECTIVE DATE: November 21, 1981.

FOR FURTHER INFORMATION CONTACT: Additional information can be obtained from Dr. William J. Gartland, Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205. (301) 496–6051.

SUPPLEMENTARY INFORMATION: I am promulgating today several actions under the NIH Guidelines for Research Involving Recombinant DNA Molecules. These were reviewed and recommended for approval by the Recombinant DNA Advisory Committee (RAC) at its meeting on September 25–26, 1980. In accordance with Section IV–E–1–b of the NIH Guidelines, I find that these actions comply with the Guidelines and present no significant risk to health or the environment.

This announcement provides background information on the actions including an analysis of correspondence concerning these actions received up to Octoiber 31, 1980.

Immediately following this announcement, there appears in the **Federal Register** a copy of revised NIH Guidelines for Research Involving Recombinant DNA Molecules. These revised Guidelines differ from the previous version of the Guidelines promulgated on January 29, 1980 (45 FR 6724) by incorporating within them: changes in the Guidelines which were recommended at the RAC meeting of March 6–7, 1980, and promulgated on April 14, 1980 (45 FR 25366); changes in the Guidelines which were recommended at the RAC meeting of June 5–6, 1980, and promulgated on July 29, 1980 (45 FR 50524); and changes in the Guidelines which were recommended at the RAC meeting of September 25–26, 1980 and which are discussed in this announcement.

I. Proposal To Introduce Genes Cloned in E. Coli K–12 into Arabidopsis Plants Through the Use of Agrobacterium Tumefaciens Carrying an E. Coli/Ti Hybrid Plasmid Vector

In a letter dated March 21, 1980, Dr. Donald J. Merlo of the University of Missouri-Columbia, requested an evaluation of the containment appropriate for the following protocol:

A. A hybrid vector, constructed from the *E. coli* plasmid pBR325 and the origin of replication and transfer genes of *Agrobacterium tumefaciens* plasmid Ti, will be cloned in *E. coli* K–12.

B. *Arabidopsis* DNA will be introduced into the *E. coli*/Ti hybrid plasmid and clone in *E. coli* K–12.

C. The thiamine gene of *E. coli* will bed introduced into the *E. coli*/Ti vector carrying *Arabidopsis* DNA and cloned in *E. coli* K–12.

D. The hybrid plasmid into which *Arabidopsis* DNA and the thiamine gene have been ligated will be transformed into *Agrobacterium tumefaciens.*

E. *Agrobacterium tumefaciens* will be used to introduce the *E. coli*/Ti plasmid vector carrying the *E. coli* Thiamine gene and *Arabidopsis* DNA into *Arabidopsis* plants.

The RAC discussed Dr. Merlo's proposed protocol at the June 5–6, 1980 meeting. The RAC stated that the first three steps of the proposed experiment are covered by Section III–O of the Guidelines. It was agreed that steps D and E are covered by Appendix E and that P3 containment is indicated. However, as the proposed inserted sequences were well characterized, and as *Arabidopsis* DNA will be returned to *Arabidopsis*, the RAC suggested that containment for steps D and E could be lowered to P2.

Since this had not been published in the **Federal Register** for comment prior to the June 5–6, 1980, RAC meeting, this proposed lowering would, however, have to be published in the **Federal Register** for thirty days of comment and re-considered at the September 25–26, 1980 meeting.

In a letter dated July 22, 1980, Dr. Merlo described the proposed experiments in greater detail and requested that the containment conditions for steps D and E be lowered at least to P2 and preferably to Pl.

Dr. Merlo's proposal was published in the August 21, 1980, **Federal Register** (45 FR 55924). No comments were received during the thirty day comment period. The RAC, at its September 25–26, 1980 meeting, once again discussed this issue. A motion to approve the experiments at the P2 level of containment failed by a vote of 4 in favor, 9 opposed, with 3 abstentions. Then, by a vote of 12 in favor, 2 opposed, with 3 abstentions, the RAC recommended approval of the proposed experiments under Pl containment.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

II. Request for Certification of a Bacillus Stearothermophilus Derived Plasmid as the Vector Component of an HV1 Bacillus Subtilis Host-Vector System

Dr. David B. Wilson of Cornell University, in a letter dated May 12, 1980, requested certification of a *Bacillus stearothermophilus* derived plasmid, pAB124, as the vector component of an HV1 *Bacillus subtilis* host-vector system. The HV1-certified *Bacillus subtilis* strain RUB331 would be used as the host component.

The RAC discussed this proposal at the June 5–6, 1980 meeting. It was noted that *Bacillus stearothermophilus* is a non-pathogenic thermophile found in soil near hot springs, on compost, etc. In addition, plasmid pAB124 can be transformed into and maintained in *Bacillus subtilis* by natural physiological methods; pAB124 might, therefore, be considered a plasmid indigenous to *Bacillus subtilis.* The committee agreed that this proposal should be published in the **Federal Register** for comment and acted upon at the September 25–26, 1980 meeting.

Dr. Wilson's proposal was published for thirty days of comment in the August 21, 1980, **Federal Register** (45 FR 55925). No comments were received during this period. The RAC once again discussed the proposal at the September 25–26, 1980 meeting. The RAC recommended approval of the proposal by a vote of 18 in favor, none opposed.

I accept this recommendation, and text has been added to Appendix D of the Guidelines indicating this.

III. Request for Certification of a Bacillus Subtilis Strain as the Host Component of an HV2 Host-Vector System

On March 28, 1980, Dr. William Burke, Jr., of Arizona State University, requested certification of *Bacillus subtilis* strain ASB298 as the host component of an HV2 host-vector system. Dr. Burke's request appeared in the **Federal Register** of April 30, 1980 (45 FR 28907). No comments were received during this period. Additional information was submitted by Dr. Burke in a letter dated May 13, 1980.

Dr. Burke's proposal was evaluated by an *ad hoc* working group and subsequently presented to the RAC at the June 5–6, 1980 meeting. One RAC

reviewer said the working group was satisfied with the data presented, and said the single issue not addressed by Dr. Burke concerned the possibility of transfer of genetic information from ASB298 to other *Bacilli* by transformation. After some discussion of this point, the RAC deferred consideration of the proposal pending the submission of additional information on transformation frequencies in soil.

In an addendum dated September 8, 1980, Dr. Burke supplied information on the persistence of ASB298 in sterile soil, and on the frequency of genetic exchange in both soil and under optimized laboratory conditions between strain ASB298 and highly transformable *B. subtilis* strains.

A second notice concerning Dr. Burke's request subsequently appeared in the **Federal Register** of August 21, 1980 (45 FR 55925). No comments on this request were received by the NIH.

The RAC reconsidered this request at its September 25–26, 1980 meeting. On the basis of the strain's characteristics and the frequency of transformation in soil, the RAC recommended, by a vote of 14 in favor, none opposed, and 2 abstentions, that strain ASB298 be certified as the host component of a *B. subtilis* HV2 host-vector system. The RAC specified that ASB898 be used with plasmids certified as the vector components of HV1 *Bacillus subtilis* host-vector systems.

I accept this recommendation, and text has been added to Appendix D of the Guidelines indicating this.

IV. Proposed Amendments Of Section IV–E–2.

In a letter dated April 10, 1980, Dr. Irving Johnson of Eli Lilly and Company, submitted several proposals to amend the Guidelines. One of these proposals would have amended Section IV–E–2 to include the following language:

"Appropriate representatives of industry shall also be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

This proposal appeared in the **Federal Register** of April 30, 1980 (45 FR 28908). Two comments were received during the thirty day comment period. One commentator stated that:

"Any attempt to add industrial expertise to the committee * * * is to convert the committee to a self-appointed consensus standards organization."

Another commentator said, "We strongly object to (this) proposal * * *"

The RAC considered this proposal at the June 5–6, 1980 meeting. The committee agreed that, while it would be desirable to have a member with expertise in fermentation technology appointed to RAC, language specifying "representatives of industry" was inappropriate. The RAC agreed that the following amended language should be published for comment and considered at the September 1980 meeting:

"Members should be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

In a letter dated July 25, 1980, Dr. Johnson requested that his original language amending Section IV–E–2 be reconsidered at the September 1980 meeting. Dr. Johnson's proposed language and the language constructed by the RAC both appeared for public comment in the **Federal Register** of August 21, 1980 (45 FR 55928). One comment was received during the thirty day comment period:

"We have long held the view that the RAC membership should include persons versed in scientific aspects of industrial microbiology. As with the rest of the Committee, they should be selected for the excellence of their qualifications. Such persons would complement the present technical expertise of RAC by providing knowledge in areas of fermentation technology and large-scale industrial applications. This expertise differs from that of safety engineers and is concerned with basic biological problems of large-scale technology * * *"

The proposals to amend the language of Section IV–E–2 were discussed by the RAC at the September 25–26, 1980 meeting. At that time, the RAC again felt that appointing fermentation technology expertise to RAC was desirable, but that the proposal language specifying appointment of "representatives of industry" was inappropriate. By a vote of 13 in favor, 2 opposed, and 2 absentions, the RAC recommended approval of the following language:

"Members should be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production."

I accept the intent of the RAC recommendation that RAC "members should be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production." I do not believe that it is necessary to change the NIH Guidelines or the RAC Charter in order to assure that the intent of the RAC be honored. This can be accomplished through appointment of one or more individuals with appropriate expertise to fill vacancies that periodically occur in RAC membership.

V. Request to Clone Schizosaccharomyces Pombe DNA in Schizosaccharomyces Pombe Using Hybrid Plasmid Vectors

Dr. Benjamin Hall of the University of Washington requested permission to clone *Schizosaccharomyces pombe* DNA in *Schizosaccharomyces pombe* using approved HV1 *Saccharomyces cerevisiae/E. coli* hybrid plasmids as vectors. Dr. Hall pointed out that *Schizosaccharomyces pombe* has been the subject of intense genetic studies in the laboratory and has been used to ferment beverages for human consumption. He requested that P1 be established as the level of physical containment.

This proposal appeared in the August 21, 1980, **Federal Register** (45 FR 55929). No comments were received during the thirty day comment period.

The RAC discussed this proposal at its September 25–26, 1980 meeting, and recommended approval by a vote of 17 in favor, none opposed.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

VI. Proposed Amendment of Appendix E of the Guidelines

In a letter dated July 28, 1980, Dr. Fritz Reusser of The Upjohn Company requested that two sections of Appendix E be amended. These sections currently read as follows:

• "*Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K–12 and *Streptomyces coelicolor* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions.

• "*Streptomyces coelicolor* can be used as a host for the cloning of DNA derived from *subtilis, E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis* under P2 conditions, using as a vector any plasmid indigenous to *Streptomyces coelicolor* or able to replicate in that host by natural biological mechanisms."

The proposed revision would permit additional *Streptomyces* species to be utilized in these experiments. The species to be added are already included in Appendix A, Sublists C and D, as two groups of *Streptomycetes* known to exchange DNA by physiological means. Dr. Reusser noted that these species are not pathogenic for humans, animals or plants. The proposed revised sections of Appendix E would read as follows:

• "*Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning

of DNA derived from *E. coli* K–12 and *Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus,* and *S. venezuelae* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions.

• "*Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus,* and *S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis,* under P2 conditions, using as vectors any plasmids indigenous to these *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms."

The proposed appeared in the **Federal Register** of August 21, 1980 (45 FR 55928). No comments were received concerning the proposal during the thirty day comment period. The RAC evaluated the proposal at the September 25–26, 1980 meeting, considering and voting separately on each of the two sections proposed to be amended.

With regard to the first section, by a vote of 14 in favor, none opposed, and 3 abstentions, the RAC recommended that the proposed amended language be adopted, i.e.:

• "*Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K–12 and *Streptomyces coelicolor S. aureofaciens, S. rimosus, S. griseus, S. cyaneus, and S. venezuelae* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions."

I accept this recommendation, and this section of Appendix E has been so amended.

The RAC then considered the second section proposed to be amended. During the discussion it was suggested that the language be modified to specify the use of "nonconjugative" plasmid vectors. By a vote of 15 in favor, none opposed, and 2 abstentions, the RAC recommended approval of the following modified language:

• "*Streptomyces coelicolor S. aureofaciens, S. rimosus, S. griseus, S. cyaneus, and S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis,* under P2 conditions, using as vectors any nonconjugative plasmids indigenous to these *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms."

Subsequent to the RAC meeting, two comments on this amended language were received by the NIH.

One commentator wrote that the proposal:

"* * *would have the effect of reversing a previous decision by the RAC and increasing the level of containment required for experiments that have been permitted under P2 conditions for some time. Thus, the amendment is a 'major action'; it does not seem appropriate that it be made part of the Guidelines in the absence of a 30-day period providing an opportunity for workers in the field and others to offer comments on it.

"It should also be noted that the concept of 'conjugative' versus 'non-conjugative' plasmids has been derived from enteric bacteria and may not apply to a wide variety of other bacterial species. For example, the mechanism for genetic exchange in *Streptomyces* species is not fully understood; it may be a consequence of hyphal fusion that permits transfer of plasmid and chromosomal genes. If this is true, it may never be possible to isolate a 'non-conjugative' plasmid *per se*' * *"

"Since all of the *Streptomyces* species that exchange genetic information with *S. coelicolor* are free-living non-pathogenic soil organisms—and since the change in the Guidelines originally proposed under Action 916 involved genes from only non-pathogenic, well-characterized donor bacteria, I urge your approval of the original proposal without the amendment."

The other commentator wrote:

"I would like to see this amended proposal published for public comment and put to a second vote at the next RAC meeting before you approve this action. The amended proposal failed to receive sufficient consideration of the following points. First of all, in no previous actions regarding *Streptomyces* species was any consideration given during the deliberations to establish levels of physical containment as to whether the vectors were capable of mediating exchange of genetic information. Therefore, this action by the RAC is more restrictive than previous actions and, in my opinion, is inconsistent with the intent of the RAC. In fact, experiments can be carried out at P2 levels of containment in which *B. subtilis, E. coli* K–12, or *S. aureus* vector DNA is cloned into *S. coelicolor* hosts using vectors capable of self-mediated exchange in accordance with Appendix E of the current NIH Guidelines.

"Second of all, the use of the term 'non-conjugative' is inappropriate because the actinomycetes do not exchange genetic information by the process of conjugation as it is known for eubacteria. Rather actinomycetes exchange genetic information by heterokaryosis. In some experiments, the frequency of genetic exchange can be altered by the presence of plasmids and some plasmids can mediate their own transfer to new hosts.

"I feel that to restrict cloning in the actinomycetes to using 'non-conjugative' or non-self-transmissible vectors would be unduly harsh and would severely restrict research with this valuable group of organisms. Considering the level of potential hazard (which I consider minimal) and the potential benefit (which I view as considerable), P2 containment is more than sufficient protection regardless of the vectors employed."

Taking into consideration these comments, I am deleting the requirement for use of nonconjugative plasmids in these systems. If anyone wishes to propose a requirement for use of nonconjugative plasmids in these systems, it will be published for comment in the **Federal Register** as a proposed major action for consideration at a future RAC meeting.

Accordingly, the relevant section of Appendix E has been amended to read as follows:

• "*Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus,* and *S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli* K–12, or from *S. aureus* vectors that have been approved for use in *B. subtilis,* under P2 conditions, using as vectors any plasmids indigenous to these *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms."

VII. Request for Consideration of a Proposal to Clone the Tox A Gene of Staphylococcus Aureus

In a memorandum dated July 29, 1980, Drs. Alan G. Barbour and Leonard W. Mayer of the Laboratory of Molecular Structure and Functions, Rocky Mountain Laboratory, National Institute of Allergy and Infectious Dieases, requested an assessment of the containment levels appropriate for cloning the *Staphylococcus aureus* pyrogenic exotoxin type A (Tox A). Drs. Barbour and Mayer indicated that they would prefer to clone the Tox A gene in an HV2 *Bacillus subtilis* host-vector system (since one of the activities of Tox A is enhancement of the toxicity of *E. coli* endotoxin) but, if no HV2 *B. subtilis* host-vector system were available, they requested permission to clone the Tox A gene in an EK2 *E. coli* K–12 host-vector system.

The RAC took this request under consideration at the September 25–26, 1980 meeting. The major determination for the RAC to make was whether Tox A is a "potent toxin" under Section I–D–2 of the Guidelines. It was noted that the lethality of Tox A for animals is not great. The RAC by a vote of 17 in favor, none opposed, recommended that the cloning of the Tox A gene be permitted under P3 containment with either an HV2 *B. subtilis* or an EK2 *E. coli* K–12 host-vector system.

The scientists who submitted this request, noting that one of the activities of Tox A is enhancement of the toxicity of *E. coli* endotoxin, said they preferred to work in *B. subtilis* rather than *E. coli*

if an HV2 *B. subtilis* host-vector system were available. Since an HV2 *B. subtilis* system is in fact now certified (see item III of this announcement) I am approving the cloning of the *Staphylococcus aureus* Tox A gene in an HV2 *Bacillus subtilis* host-vector system at P3 containment. Text has been added to Appendix E indicating this.

VIII. Request for Permission to Transform Chlamydomonas Reinhardi With E. Coli/Saccraromyces Cerevisiae Plasmids.

The RAC at its September 25–26, 1980 meeting considered similar requests from Dr. John Carbon of the University of California, Santa Barbara, and Dr. Stephen Howell of the University of California, San Diego, to introduce *E. coli-Saccharomyces cerevisiae* hybrid plasmids containing defined DNA segments into *Chlamydomonas reinhardi* under P2 conditions. The host is a eukaryotic, unicellular photosynthetic green algae. *C. reinhardi* is non-pathogenic, produces no known toxin, and is not known to exchange genetic information with other organisms.

This proposal was published for comment in the **Federal Register** of August 21, 1980. No comments were received during the thirty day period for comment.

A motion to approve these experiments at the P2 level of containment was passed by a vote of 16 in favor, none opposed, and one absention.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

IX. Request For Permission To Transform Candida Albicans With E. Coli-S. Cerevisiae Plasmid.

The following notice appeared in the **Federal Register** of August 21, 1980:

Request for permission to transform Candida albicans with E. coli-S. cerevisiae plasimids. Dr. P. T. Magee of Michigan State University, and Dr. W. LaJean Chaffin of Texas Tech University, have requested consideration of the appropriate containment level for the return of *Candida albicans* DNA to the host of origin. The *Candida albicans* DNA will be cloned in *E. coli* K–12 or in *Saccharomyces cerevisiae* employing a hybrid plasmid vector derived from *E. coli* K–12–S. *cerevisiae* or the yeast 2 micron plasmid.

During the thirty day comment period, no responses were received.

The RAC discussed this proposal at its September 25–26, 1980 meeting. It was noted that *Candida albicans* is a normal inhabitant of the flora of man. It can be a pathogen in compromised individuals but does not produce a toxin. This is basically a return of DNA to host of origin type of experiment, with the intermediate host being either *E. coli* K–12 or *Saccharomyces cerevisiae.*

The RAC by a vote of 14 in favor, 1 opposed, and 3 absentions recommeded approval of this proposal at the P2 level of containment.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

X. Proposal To Transform Haemophilus Influenzae With E. Coli/H. Influenzae Hybrid Plasmid

The RAC at its September 25–26, 1980 meeting considered a proposal from Dr. Hamilton Smith of the John Hopkins University to insert an *E. coli* Tn10 tet^R gene into a naturally-occurring *Haemophilus* plasmid and to use the hybrid plasmid to transform *H. influenzae* Rd, a nonpathogenic strain. It was noted that a tetracycline resistance gene found naturally in *Haemophilus* is genetically related to the Tn10 tet^R gene of *E. coli.* A motion to approve these experiments at the P1 level of containment was passed by a vote of 12 in favor, none opposed, with 7 abstentions.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

XI. Proposed Exemption For Streptococcus Sanguis And Streptococcus Pneumoniae

A request submitted by Dr. Walter Guild of Duke University Medical Center that *Streptococcus sanguis* and *Streptococcus pneumoniae* be considered as natural exchangers of DNA under the exemption category of Section I–E–4 and Appendix A of the Guidelines was considered by the RAC. This proposal was published for comment in the **Federal Register** of August 21, 1980. No comments were received during the 30 day period for comment prior to the September 25–26, 1980 meeting.

The evidence presented by the investigator demonstrated that these organisms exhange genetic material in both directions by the natural process of transformation. The RAC recommended by a vote of 16 in favor, none opposed, and 1 absention that a new sublist be added to Appendix A of the Guidelines exempting recombinant DNA experiments between *Streptococcus sanguis* and *Streptococcus pneumoniae.*

I accept this recommendation and these two organisms have been added as sublist F of Appendix A.

XII. Request For Permission To Incorporate Recombinant DNA In Zymomonas Mobilis

The RAC considered a request from Drs. B. Montenecourt and D. Eveleigh of Rutgers University to permit the cloning of DNA derived from *Pseudomonas* strains that are non-pathogenic to animals or plants in an *E. coli* K–12 host, followed by transfer of the recombinant DNA into *Zymomonas mobilis.*

This proposal was published for comment in the **Federal Register** of August 21, 1980. No comments were received during the 30 day comment period.

The RAC by a vote of 17 in favor, none opposed and with no abstentions recommended approval at the P2 level of containment for the proposed experiments.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

XIII. Request To Transform Protoplasts of Streptosporangium With Recombinant DNA

The RAC at its September 26, 1980 meeting considered a proposal to transform protoplasts of *Streptosporangium* with a hybrid plasmid containing pBR322 plus a *Streptosporangium* plasmid into which have been incorporated specified DNA segments from *Streptomyces* species or an HV1 approved *Bacillus subtilis* cloning vector.

It was noted that members of the *Streptosporangium* genera are soil bacteria and have never been implicated in any human, animal, or plant diseases. Further, these nutritionally fastidious organisms have not been reported to produce toxins harmful to humans.

The motion to approve these experiments at the P2 level of containment was passed by a vote of 17 in favor, none opposed, and 1 abstention.

I accept this recommendation, and text has been added to Appendix E of the Guidelines indicating this.

XIV. Proposed Revision of Subsections of Section III–C–1–e

A notice appeared in the **Federal Register** of January 31, 1980 concerning proposed revision of Section III–C–1–e, and its subsections. It was recommended that Section III–C–1–e, III–C–1–e–(1), III–C–1–e–(1)–(a), and III–C–1–e–(1)–(b), of the Guidelines be changed and that a new Section III–C–1–e–(1)–(c) be added. Section III–C–1–e–(2) would remain unchanged. The RAC, at its March 6–7, 1980 meeting, recommended adoption of Section III–C–

1–e, III–C–1–e–(1), and III–C–1–e–(1)–(a) as published in the **Federal Register** of January 31, 1980, with certain modifications in Section III–C–1–e–(1)–(a).

The Director, NIH, accepted this recommendation and promulgated the following sections in the **Federal Register** of April 14, 1980:

"III–C–1–e. All Viral Vectors.

"III–C–1–e–(1). Other experiments involving eukaryotic virus vectors can be done as follows:

"III–C–1–e–(1)–(a). Recombinant DNA molecules containing no more than two-thirds of the genome of any eukaryotic virus [all viruses form a single Family (36) being considered identical (50)] may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of the genome."

At its March 6–7, 1980 meeting, the RAC voted to defer consideration until the June 5–6, 1980 meeting of the new Sections III–C–1–e–(1)–(b) and III–C–1–e–(1)–(c) as proposed in the **Federal Register** of January 31, 1980, and requested that a working group develop additional information. Accordingly, a working group met on May 13, 1980, during the annual meeting of the American Society for Microbiology in Miami Beach, Florida. The report of the working group was considered briefly at the June 5–6, 1980 RAC meeting when it was decided that it would be considered again by the RAC at its September 25–26, 1980 meeting.

The Working Group discussed the question of the appropriate containment conditions for experiments involving recombinant DNA molecules containing less than two-thirds of the genome of any eukaryotic virus which may be rescued with helper virus. On the basis of the consensus of the virologists, the following recommendation was proposed, as a revision of Section III–C–1–e–(1)–(b) of the Guidelines:

"III–C–1–e–(1)–(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with helper virus using P2 containment if wild type strains of the virus are CDC Class 1 or 2 agents, or using P3 containment if wild type strains of the virus are CDC Class 3 agents (1)."

This proposal was published for comment in the **Federal Register** of August 21, 1980. During the thirty day comment period no responses were received.

At the September 25–26, 1980 meeting, the RAC discussed this proposal again. Some members of the committee expressed concern whether the rescue of a defective recombinant virus with helper virus could result in the formation of a virus with an altered host specificity or increased pathogenicity. It was noted that the working group of virologists previously discussed this point, and reached the conclusion that the recombinant virus would pose no greater biohazard than the wild type virus used as a helper. The consensus of the working group was that the level of containment required for rescue of the recombinant virus should correspond to the same level of biohazard as the helper virus as determined by the CDC classification.

The motion to accept the proposed revision of subsection III–C–1–e–(1)–(b) was passed by a vote of 10 in favor, 5 opposed, and 1 abstention.

I accept this recommendation, and Section III–C–1–e–(1)–(b) has been so modified. This modification justifies two further changes in the Guidelines. First, new text has been added to Section III–C–1 to advise the reader of the relationship of the subsections of Sections III–C–1–a, III–C–1–b, III–C–1–c, and III–C–1–d of the Guidelines to the subsections of Section III–C–1–e. Also, Table IV of the Guidelines has been eliminated.

XV. Proposed Containment For Cloning Between Members of the Actinomycetes Group

Dr. Dean Taylor of the Smith Kline and French Laboratories, proposed that the third entry in Appendix E of the Guidelines be modified to read:

P2 physical containment shall be used for DNA recombinants produced between members of the *Actinomycetes* group except for those species which are known to be pathogenic for man, animals, or plants.

This proposal was made previously by the RAC Working Group on Prokaryotic Host-Vectors Other Than *E. coli* and appeared in the **Federal Register**, April 13, 1979 (44 FR 22316). The RAC considered the proposal at its May 21–23, 1979 meeting and recommended to restrict this so that it did not include the entire *Actinomycetes* group but rather only the genera *Streptomyces* and *Micromonospora*. The Director, NIH, accepted this recommendation and the action was published in the **Federal Register**, July 20, 1979 (44 FR 42916), and appears as the third entry in Appendix E of the Guidelines.

This proposal was published for comment in the **Federal Register** of August 21, 1980. During the 30 day comment period, no comments were received.

During the RAC discussion of this proposal it was noted that the family of *Actinomycetes* include many genera that are not pathogenic for man, animals, or plants. The microorganisms in this group are mainly found in soil, and are of medical and industrial importance. They produce ninety percent of the antibiotics used in medicine. Although some members of the group are parasitic, most do not cause disease, or are marginal pathogens.

A motion to restrict the proposal to members of the *Actinomycetes* group which are known not to be pathogenic for man, animals, or plants, failed by a vote of 3 in favor, 11 opposed, with 3 abstentions.

A motion to accept the proposal as published in the **Federal Register** on August 21, 1980, passed by a vote of 16 in favor, 1 opposed, with 1 abstention.

I accept this recommendation, and the third entry in Appendix E of the Guidelines has been so amended.

XVI. Changes in Registration Requirements

Dr. Maxine Singer, National Cancer institute, National Institutes of Health, proposed a series of changes in the administrative requirements specified by the Guidelines. The proposal would elimnate NIH review, registration, and approval for all experiments assigned containment conditions in the Guidelines.

In submitting the proposal, Dr. Singer stated:

When the Guidelines were first promulgated, many arguments appeared to dictate a complex system of review and approval prior to the initiation of experiments. Regardless of how one views the wisdom of the earlier decisions, the multi-level system with its emphasis on central review at the NIH is now clearly unnecessary, inhibitory, expensive, and conterproductive in relation to the respect accorded the containment recommendations. By now, Institutional Biosafety Committees have accumulated sufficient expeience with and knowledge of the Guidelines to operate as independent review groups. Safety Officers now function in many research institutions. Furthermore, the good laboratory practices mandated by the Guidelines have become routine practice in many laboratories. Indeed, confidence in the efficiacy of Guidelines has been based primarily on compliance by individual investiators within their own laboratories. The administrative changes that accompanied the revised containment levels for experiments with *E. coli* K–12 and *S. cerevisiae* earlier this year recognized that central review and approval were no longer necessaryily desirable or useful. The present proposal is an extension of that idea.

In brief, the proposal eliminates central review, registration, and approval for all

experiments assigned containment conditions in the Guidelines. Investigators would be required to notify their Institutional Biosafety Committees prior to the initiation of experiments and those committees would be responsible for reviewing the registration documents for consistency with the provisions of the Guidelines and advising the investigators of necessary changes. No Memoranda of Understanding would be required. IBCs and investigators would still be expected to petition the ORDA regarding experiments not explicitly covered by the Guidelines, experiments requiring case-by-case review and experiments involving exceptions to the provisions (or prohibitions). The NIH and the RAC would function as policy making elements with responsibility to change the Guidelines as required, to evaluate containment requirements for experiments not explicitly covered by the Guidelines, and for consideration of exceptions.

Seven years have passed since the scientific community first raised questions regarding recombinant DNA experiments. The ignorance of those early years has been supplanted with a wealth of experience and information. Just as the early stringent Guidelines were promptly adopted in response to the ignorance, we must now respond just as promptly to the current more realistic appraisals.

The proposed revisions were published in the **Federal Register** of August 21, 1980, for comment, as Item 8 of the notice. Specific changes were proposed in many different places in the Guidelines, specified under subheadings "A" through "X" within Item 8.

Seventeen letters were received commenting on the proposal. Sixteen commentators supported its adoption. One commentator stated:

Maxine Singer's initiative (**Federal Register**, August 21, 1980) to have the NIH Guidelines fall primarily under local control is both wise and timely. Decisions about scientific research are best left to the investigtors both for efficiency and as a matter of fundamental principle. Only in exigency, should this ideal be violated. Perhaps we've passed through such an occasion with R–DNA research. However, it is now the time to return to the more normal situation. Since work with recombinant DNA has proven to be very safe and very important, dual review at the national and local levels serves no good purpose.

As one involved from the beginning in the R–DNA issue, I've always felt that the 'Guidelines' should be guidelines; they turned into regulations. With the proposed change, they will revert and also the unfortunate quasi-regulatory role that the NIH has had to play will begin to disappear.

Another commentator said:

I think the IBC mechanism has proven its effectiveness in interpreting and enforcing the letter and intent of the Guidelines governing recombinant DNA research. I do not see how the practices of submission, obtaining and recording, etc., of MUA's particularly the requirements for annual submissions of MUA's for each continuing NIH grant or for applications for new grants, contributes to the safety of research. An updated statement of work in progress from each investigator to the IBC's would be significantly simpler to administer and would ensure as much real safety as the present system.

Another observed:

I agree with her that the function of review, registration, and monitoring of research workers falling into categories explicitly mentioned in the Guidelines can be effectively administered by the local Institutional Biosafety Committees. This would reduce the workload for ORDA allowing it to spend more time determining policy and revising the Guidelines according to requests or to new data. It would simultaneously ease the bureaucratic workload for the researcher without jeopardizing the effectiveness of the Guidelines. Not only are the procedures described in the Guidelines now standard practice among most laboratories, as pointed out by Dr. Singer, but I feel that the local Institutional Biosafety Committees are in much better position to evaluate compliance with the Guidelines. Because of their past experience with the Guidelines, I feel that these committees are ideally suited to take over this important administrative role.

One commentator made the following observation about Institutional Biosafety Committees:

Institutional Biosafety Committees clearly seem able to operate as independent review groups, and the laboratory practices set forth in the Guidelines have now become part of the standard operating procedures of laboratories working in the field. In other fields of biological research, safety procedures are implemented entirely at the local level, even when known biohazards are involved. It is no longer necessary to have a unique multi-level system in an area where seven years of extensive experience has shown the absence of a unique hazard.

Another commentator said the following about central review:

Elimination of central review at NIH for experiments classified in the Guidelines will save a great deal of time for both the investigator and the reviewers at NIH. I believe the experience of the past six years justifies a change toward an administrative arrangement long successfully practiced in the handling of proven microbial pathogens, namely national guidelines enforced by local biosafety committees.

One commentator made the following observation about the current system of NIH review:

The present complex system of review on both local and Federal levels is no longer necessary. It is wasteful of time, effort, and money. It is, in fact, counterproductive because bureaucratic requirements seen by investigators to be clearly unnecessary lead to disrespect for regulations that should be respected. Institutional Biosafety Committees should, in my opinion, be fully capable of monitoring laboratory practices and containment levels specified by the Guidelines.

Another letter included the following remark:

I strongly endorse the proposed changes because I believe that the risks of this research are now clearly minimal and because the bureaucracy and consequent wasted time are truly detrimental to progress in an area that promises to be of great benefit to mankind.

The Chairman of one IBC reporting the unanimous endorsement of his committee stated:

We feel strongly that safety is solely a function of the effectiveness of the local institutional practices, on the part of the PI's and the IBC, in complying with and enforcing the Guidelines. The submission of MUAs adds nothing but expensive and time-consuming paperwork.

The one letter against the proposal stated the following:

At present, we do not believe that there are good reasons for changing the registration requirements. Although present procedures demand some extra paperwork, they permit centralized keeping of files which could be of significant use in reviewing the extent of work on recombinant DNA and its possible hazards. Somebody should know what is going on.

The RAC discussed the proposal at length at its September 25–26, 1980 meeting. Dr. Singer, who attended this portion of the meeting, summarized the proposal and presented her arguments for adopting it. It was noted that the proposal has two major aspects, which could be acted upon separately. The first aspect is that it would eliminate NIH review of protocols for which containment levels are specified by the Guidelines. The second aspect is that the proposal would eliminate the requirement for pre-review by the local IBC prior to initiation of all experiments for which containment levels are specified by the Guidelines (Prior review is already not required for most experiments). Some members of the RAC felt that adoption of the proposal should be delayed until a broad-based survey of the functioning of IBCs is completed. Some members of the RAC felt that pre-review of all experiments by the IBC should not be eliminated at the present time. The RAC then voted separately on a four-part motion proposed by Dr. Gottesman.

The RAC voted 15 in favor, 3 opposed, with no abstentions, to eliminate the requirement for NIH review of IBC decisions on any experiments for which containment levels are specified in the Guidelines.

The RAC voted 12 in favor, 5 opposed, with 1 abstention, to defer consideration

of eliminating pre-review of experiments by the IBCs until the frequency of principal investigator error in selecting the appropriate containment levels has been determined.

The RAC voted 17 in favor, none opposed, with 1 abstention, that IBCs keep records of recombinant DNA research done in their institution, including a record of the frequency of errors in classificaion of experiments by the principal investigator.

The RAC voted 15 in favor, 3 opposed, with no abstentions, that IBCs no longer need register with NIH recombinant DNA experiments for which containment levels are specified in the Guidelines.

I accept these recommendations.

The four-part motion passed by the RAC indicated the intent of the RAC to recomment acceptance of certain concepts in the proposal by Maxine Singer (i.e., elimination of the requirement for IBC registration with, and NIH review of, experiments for which the containment levels are specified in the Guidelines), but to defer recommendation of other concepts (i.e., the proposal to eliminate pre-review by the IBC for these experiments).

As noted earlier, the proposal by Maxine Singer published as Item 8 in the **Federal Register** of August 21, 1980, consisted of proposed changes in many different places of the Guidelines, specified under subheadings "A" to "X". Following the RAC meeting, I asked a four-person group to revise these proposed changes in order to translate the four-part general motion of the RAC into specific Guideline changes. The group (consisting of Dr. Bernard Talbot, my Special Assistant, Dr. William Gartland, Director of the Office of Recombinant DNA Activities, Dr. Maxine Singer, originator of the proposal, and Dr. Susan Gottesman, RAC member and originator of the four-part motion passed by the RAC) unanimously agreed on wording which translates the intent of the RAC into specific changes in the Guidelines. I have accepted their recommendations.

The result is that some of the changes "A" through "X" originally proposed by Dr. Singer have been accepted as proposed; some have been rejected, thereby leaving the relevant portions of the Guidelines unchanged; and some have been further modified so that particular sections now are different from both the Guidelines prior to the Singer proposal and from the Singer proposal.

Specifically, the sections where the changes proposed by Dr. Singer have been accepted are: "E"—Deletion of Section IV–C–3; "F"—Deletion of Sections IV–D–1–C and IV–D–1–C–(1) through IV–D–1–C(5); "G"—Deletion of Section IV–D–1–d; "H"—Amendment of Section IV–D–2–f; "I"—Amendment of Section IV–D–2–h; "K"—Amendment of Section IV–D–3–b; "L"—Amendment of Section IV–D–3–f; "P"—Amendment of Section IV–D–5–c–(3); "U"—Deletion of Section IV–E–3–b, IV–E–3–c–(1), IV–E–3–c–(2), and IV–E–3–c–(3); "V"—Deletion of Section IV–E–4–a; and "W"—Deletion of Sections IV–F–1 and IV–F–2.

The proposed changes which have been rejected thus leaving the Guidelines as they were previous to the Singer proposal are: "Q"—Heading of Section IV–D–5–d; "R"—Heading of Section IV–D–5–e; and "T"—Proposed deletion of Section IV–E–1–b–(3)–(e).

The proposed changes which have been further modified so that the sections now read differently from the Guidelines prior to the Singer proposal and from the Singer proposal are: "A"—Section III; "B"—Section III–0; "C"—Last sentence of Section III–A–3–a; "D"—Last sentence of Section III–A–3–b; "J"—Section IV–D–3–a; "M"—Section IV–D–5–a–(1); "N"—Section IV–D–5–b–(4); and "O"—Section IV–E–5–b–(5).

In addition, Sections IV–F–3, IV–F–4, and VI–C, dealing with registration, have been eliminated, as has the proposed Section IV–D–5–e–(6).

One consequence of these changes is that MUAs need no longer be filed with NIH and the term "MUA" has been deleted from the Guidelines.

One part of the RAC four-part motion called for IBCs to keep records of recombinant DNA research done in their institution, including a record of the frequency of errors in classification of experiments by the principal investigator. This requirement for record keeping will be specified in the revision of the Administrative Practice Supplement to the NIH Guidelines to be issued in November 1980.

During the RAC discussion, the concept of requiring the IBCs to report annually to NIH on all recombinant DNA research being done at the institution was rejected. NIH is sponsoring a meeting of IBC Chairmen on November 24–25, 1980. The issue of possibly reporting annually to NIH will be discussed at that meeting. Following that discussion, I will again review this issue.

It should be emphasized that NIH remains responsible for specifying containment conditions for all experiments not explicitly covered by the Guidelines. This includes experiments requiring case-by-case review and exceptions to the provisions or prohibitions of the Guidelines. Principal investigators must petition NIH for consideration of such proposed experiments. NIH will follow existing procedures in such cases and will notify the principal investigators as before. No such experiments are to be approved by the IBCs until containment conditions have been set by the NIH. No such experiments are to be initiated by PIs until appropriate registration documents have been submitted to and approved by the IBC. The registration documents should include the NIH statement of containment conditions.

XVII. Procedures For Review of Large-Scale Experiments

Section I–D–6 of the Guidelines prohibits "large-scale experiments (e.g., more than 10 liters of culture) with organisms containing recombinant DNA's, unless the recombinant DNAs are rigorously characterized and the absence of harmful sequences established."

Section IV–E–1–b–(3)–(d) of the Guidelines states that the Director, NIH, is responsible for "authorizing under procedures specified by the RAC, large-scale experiments (i.e., more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences."

Part VI of the Guidelines, "Voluntary Compliance," encourages institutions not otherwise covered by the Guidelines to follow the standards and procedures set forth in the Guidelines.

At its September 1979 meeting, the RAC adopted the following procedures to be followed by applicants proposing to exceed the 10-liter limit:

"Application Procedures for Large-Scale Recombinant DNA Experiments

"1. For each research project proposing to exceed the 10-liter limit, the applicant shall file a request with the NIH Office of Recombinant DNA Activities (ORDA). The request should include the following information:

"a. The Memorandum of Understanding and Agreement (MUA) submitted to the local Institutional Biosafety Committee. The MUA should include, or have appended to it, a summary paragraph which describes the proposed project in language that is comprehensible to non-specialists.

"b. A statement of the rationale for wishing to exceed the 10-liter limit.

"c. A specification of the total volume of the fermenter to be used.

"d. Evidence that the recombinant DNAs to be employed in the research have been rigorously characterized and are free of harmful sequences.

"e. A description of the applicant's laboratory practices, containment equipment, and facilities relevant to the containment of large volumes of culture.

"f. Evidence of the applicant's or applicant institution's previous experience in handling large volumes of culture. Applicants should exhibit knowledge of state-of-the-art procedures for working with large volumes of microorganisms.

"g. A description of procedures to be employed for the inactivation and disposal of large volumes of culture.

"h. A description of procedures for containing and inactivating accidental spills, should they occur.

"2. Each request submitted to ORDA shall be referred to a working group of the NIH Recombinant DNA Advisory Committee for review.

"3. Following review and approval by the working group, each request shall be submitted to the entire Recombinant DNA Advisory Committee for review.

"4. Following review and approval by the RAC, each request shall be submitted to the Director, NIH, for final review.

"5. Applications for large-scale experiments which are submitted by institutions not receiving NIH funds for recombinant DNA research shall be kept confidential (provided the institutions so desire) in accordance with the provisions of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

"These procedures may be refined or revised on the basis of discussion and action by the NIH Recombinant DNA Advisory Committee."

At recent RAC meetings, there have been extensive discussions of the role of the RAC and NIH in the review of large-scale proposals submitted by industry. (Minutes of RAC meetings are available from ORDA.) At the June 1980 meeting, the RAC passed a motion by a vote of seventeen to zero with one abstention that the following proposal be published in the **Federal Register** for consideration at the September 1980 meeting:

"The following procedures should be adopted for approval of requests to grow greater than 10 liters of organisms containing recombinant DNA. The RAC will determine if a given recombinant DNA-containing strain is rigorously characterized and the absence of harmful sequences established. Such a determination shall include specification of the containment level (P–LS). These determinations should not in any way be construed as RAC certification of safe laboratory procedures for industry scale-up. Adherence to the specified containment conditions is the responsibility of the local IBC."

This proposal was published for comment in the **Federal Register** of August 21, 1980. There it was pointed out that if the proposal were accepted, it would have the effect of changing the application procedures to read as follows:

"Application Procedures for Large-Scale Recombinant DNA Experiments

"1. For each research project proposing to exceed the 10-liter limit, the applicant shall file a request with the NIH Office of Recombinant DNA Activities (ORDA). The request should include the following information:

"a. The Memorandum of Understanding and Agreement (MUA) submitted to the local Institutional Biosafety Committee. The MUA should include, or have appended to it, a summary paragraph which describes the proposed project in language that is comprehensible to non-specialists.

"b. A statement of the rationale for wishing to exceed the 10-liter limit.

"c. Evidence that the recombinant DNAs to be employed in the research have been rigorously characterized and are free of harmful sequences.

"d. Specification of the P–LS level proposed to be used as defined in the NIH *Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules.* (**Federal Register,** April 11, 1980).

"2. Each request submitted to ORDA shall be referred to a working group of the NIH Recombinant DNA Advisory Committee for review.

"3. Following review and approval by the working group, each request shall be submitted to the entire Recombinant DNA Advisory Committee for review.

"4. Following review and approval by the RAC, each request shall be submitted to the Director, NIH, for final review.

"5. Applications for large-scale experiments which are submitted by institutions not receiving NIH funds for recombinant DNA research shall be kept confidential (provided the institutions so desire) in accordance with the provisions of the NIH Guidelines for Research Involving Recombinant DNA Molecules.

"These procedures may be refined or revised on the basis of discussion and action by the NIH Recombinant DNA Advisory Committee."

During the comment period, two comments were received.

One commentator stated:

> I regret that the RAC wishes to terminate its reviews of engineering plans for proposed private-sector DNA operations.

Another letter stated:

> We agree with the RAC proposal passed in June 1980, to exclude regulatory functions dealing with industrial scale-up. We believe that the current mission of the RAC is appropriate and that its present constitution, representing scientific and public interests, is not well suited for a regulatory role. Other bodies of the federal government already have the mandate to carry out regulatory functions and should acquire necessary resources and expertise.

The proposal was discussed at length at the September 25–26, 1980 RAC meeting.

Dr. Krimsky said that although at previous RAC meetings he had argued strongly for proposals like this, he had now changed his mind, because he had come to realize that such pre-review as the RAC has been performing is unique, and that no other agency would conduct such a review. He proposed, as an alternative proposal, that there be established a subcommittee of the RAC made up of some members of the RAC and some members of NIH staff with expertise in facilities and technologies, and that the subcommittee request representation from the Occupational Safety and Health Administration (OSHA), the National Institute for Occupational Safety and Health (NIOSH), the Center for Disease Control (CDC), and the Environmental Protection Agency (EPA). In this proposal, the subcommittee would review engineering and technology, and its review would be transmitted directly to the Director, NIH. After discussion, this alternative motion was withdrawn from consideration for the time being, with the understanding that it would be reconsidered later in the meeting.

During further discussion of the proposal as published in the **Federal Register** of August 21, 1980, Dr. Fedoroff indicated her support for eliminating RAC review of the details of physical containment for individual large-scle proposals, as is already the case for small-scale research. Dr. King indicated her support of the proposal as an acceptable compromise.

Following further discussion, the RAC voted 12 in favor, 5 opposed, with one abstention, to adopt the proposal as published in the **Federal Register** of August 21, 1980. I accept this recommendation and procedures for its implementation are published at the end of this section.

Later in the meeting, Dr. Krimsky introduced the following motion to establish a large-scale review subcommittee:

"An industrial review subcommittee of the RAC shall be established with the responsibility for advising the Director on procedures and facilities design pertaining to applications for large-scale operations.

"After the full RAC has reviewed the biological containment requirements for

a large-scale proposal, the subcommittee shall examine the applicant's plans for large-scale operations and issue recommendations to the Director on plant design, health surveillance and environmental monitoring. The Director shall advise institutions of recommended design parameters and operational procedures. The determination shall not be construed as NIH certification of industrial operations.

"The subcommittee shall invite participation from NIH's biosafety staff plus OSHA, NIOSH, CDC, FDA, EPA, and USDA."

Dr. Fedoroff expressed concern about delays if first the full RAC and then subsequently a subcommittee were to be involved in the review process. Others agreed. Dr. Gottesman said that she was opposed to continuation of review of equipment design in individual applications by either the full RAC or subcommittee. Dr. Logan of OSHA, speaking in behalf of the proposal, said that the delays need not be serious and that such subcommittee review would be beneficial. He said that OSHA does not have the legal authority to conduct pre-review of applications. Dr. Campbell said that while he opposed a subcommittee doing pre-review of individual applications, he favored a subcommittee to prepare future revisions of the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules (i.e., the definitions of Pl-LS, etc.) and to monitor how well the system is working. Dr. Gottesman proposed an amendment to Dr. Krimsky's motion to establish the subcommittee with these functions, but to do no pre-review of individual applications. An amendment to this amendment proposed by Dr. Krimsky to include subcommittee pre-review of individual applications failed by a vote of 3 in favor, 15 opposed, with no abstentions. After further amendments were adopted, the RAC passed the following motion by a vote of 15 in favor, 2 opposed, with 1 abstention:

"A large-scale review subcommittee of the RAC shall be established with the responsibility for advising the RAC on procedures and facilities design pertaining to large-scale operations, and on the performance of local IBCs in reviewing physical containment facilities.

"The subcommittee shall invite participation from NIH's biosafety staff plus OSHA, NIOSH, CDC, FDA, EPA, and USDA."

I agree with the establishment of a group to report to the RAC on recommendations for future revisions of the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules (i.e., the definitions of P1-LS, etc.) and on the performance of local IBCs in reviewing large-scale physical containment facilities. A Working Group of the RAC was established at the May 1979 meeting to develop the Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules. These recommendations were reviewed at the December 1979 and March 1980 RAC Meetings before being published in the **Federal Register** on April 11, 1980. Establishing a new "subcommittee" of the RAC to deal with this issue would require a formal change in the RAC Charter. Therefore, I favor reconstitution of the current RAC "Working Group" for this purpose. As noted above (see item IV of this announcement), the RAC has recommended that RAC "members should be chosen to provide expertise in fermentation technology, engineering, and other aspects of large-scale production." I expect that an individual with these qualifications will shortly be appointed. When this happens, I will ask that the appointee consult with the RAC Chairman and that together they decide on which additional RAC members should be appointed to the reconstituted Large-Scale Review Working Group. The RAC recommended that participation be invited from "NIH's biosafety staff plus OSHA, NIOSH, CDC, FDA, EPA, and USDA." Ideally, all Federal agencies with representatives on the RAC should be invited to participate in the deliberations of the Working Group.

In accordance with the recommendation of the RAC, the Working Group will not be involved in pre-review of individual applications. To assist in its function of advising the RAC on the performance of local IBCs in reviewing physical containment facilities and on future revisions of the Large-Scale Physical Containment Recommendations, the Working Group may request information from individual companies. Since NIH is not a regulatory agency, the intent of any such information collection will be fact-finding, to help in development of general recommendations from the Working Group to the RAC, and not for purposes of regulatory actions directed at individual companies.

In response to recommendations of the RAC, the revised application procedures for large-scale proposals are now promulgated as final, i.e.:

"Application Procedures for Large-Scale Recombinant DNA Experiments.

"1. For each research project proposing to exceed the 10-liter limit, the applicant shall file a request with the NIH Office of Recombinant DNA Activities (ORDA). The request should include the following information:

"a. The registration document submitted to the local Institutional Biosafety Committee. This should include, or have appended to it, a summary paragraph which describes the proposed project in language that is comprehensible to non-specialists.

"b. A statement of the rationale for wishing to exceed the 10–liter limit.

"c. Evidence that the recombinant DNAs to be employed in the research have been rigorously characterized and are free of harmful sequences.

"d. Specification of the P–LS level proposed to be used as defined in the NIH *Physical Containment Recommendations for Large-Scale Uses of Organisms Containing Recombinant DNA Molecules. (Federal Register,* April 11, 1980).

"2. Each request submitted to ORDA shall be referred to a working group of the NIH Recombinant DNA Advisory Committee for review.

"3. Following review and approval by the working group, each request shall be submitted to the entire Recombinant DNA Advisory Committee for review.

"4. Following review and approval by the RAC, each request shall be submitted to the Director, NIH, for final review.

"5. Applications for large-scale experiments which are submitted by institutions not receiving NIH funds for recombinant DNA research shall be kept confidential (provided the institutions so desire) in accordance with the provisions of the NIH Guidelines for Research Involving Recombinant DNA Molecules and to the extent permitted by law.

"These procedures may be refined or revised on the basis of discussion and action by the NIH Recombinant DNA Advisory Committee."

These application procedures are identical to those published for comment on August 21, 1980, except for the elimination of the term "Memorandum of Understanding and Agreement," its replacement by the term "registration document," and addition of the clause "and to the extent permitted by law" in paragraph 5. As discussed above (in item XVI of this announcement), the term "Memorandum of Understanding and Agreement" has been eliminated from the Guidelines. The revised Guidelines, in Section III, describe the

required contents of a registration document.

The revised "Application Procedures for Large-Scale Recombinant DNA Experiments" differ from the previous version (adopted at the September 1979 RAC meeting) in requiring that the application specify a "P–LS" level at which the work will be done, rather than requiring individual details of physcial containment. In other respects the Application Procedures adopted at the September 1979 RAC meeting remain identical in the revised Application Procedures promulgated today. As noted in the minutes of the September 6–7, 1979 RAC meeting, review of each large-scale proposal by the RAC, "might be at a meeting, but it also might be through mail ballot." This option will be retained.

Additional Announcements of the Director, NIH

Section IV-E-1-b-(3)-(d) of the Guidelines gives responsibility to the Director, NIH, for "authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinant DNAs that are rigorously characterized and free of harmful sequences."

Accordingly, several requests for authorization to culture, on a large-scale, recombinant DNA host-vector systems have been received and reviewed by the NIH.

I. Genentech, Inc.

On November 4, 1980, the Director, NIH, on the recommendation of the RAC, approved a request from Genentech, Inc., for the large-scale culture up to 750 liters of EK1 host-vector systems containing plasmids into which have been ligated cDNA coding for human leukocyte interferons.

This request was approved with the understanding that Genentech, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site.

The principal investigators are Drs. Michael Ross and Norm S. C. Lin. The work is to be done at the Pl–LS level of containment at the research and development facility at 460 Point San Bruno Boulevard, South San Francisco, California 94080.

II. Burns-Biotec Laboratories, Inc.

On November 4, 1980, the Director, NIH, approved requests from Burns-Biotec Laboratories, Inc., a wholly-owned subsidiary of Schering Corporation, for large-scale culture of EK1 host-vector systems containing plasmids coding for human leukocyte interferon, and certain derivatives thereof, in a 1000 liter fermentor (up to 750 liter working volume) at the Pl–LS level of containment.

The request was approved with the understanding that Burns-Biotec Laboratories, Inc., has agreed to permit an observer, designated by NIH, to visit the facilities if NIH should choose to inspect the site. The principal investigator for this project is Dr. Donald E. Baldwin. The large-scale growth of the organisms is to be carried out at plant facilities located in Elkhorn, Nebraska.

Dated: November 14, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government programs contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its announcements the number and title of affected individual programs for the guidance of the public. Because of the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "programs not considered appropriate" in Section 8–(b)–(4) and (5) of that Circular.

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Part VII

Department of Health and Human Services

National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules, November 1980

HEALTH AND HUMAN SERVICES DEPARTMENT

National Institutes of Health

Guidelines for Research Involving Recombinant DNA Molecules, November 1980

These NIH Guidelines supersede those of January 1980, and will be in effect until further notice.

Table of Contents

I. Scope of the Guidelines
- I–A Purpose
- I–B Definition of Recombinant DNA Molecules
- I–C General Applicability (see IV–B)
- I–D Prohibitions
- I–E Exemptions
- I–F General Definitions (see IV–C)

II. Containment
- II–A Standard Practices and Training
- II–B Physical Containment Levels
- II–B–1 P1 Level
- II–B–1–a Laboratory Practices
- II–B–1–b Containment Equipment
- II–B–1–c Special Laboratory Design
- II–B–2 P2 Level
- II–B–2–a Laboratory Practices
- II–B–2–b Containment Equipment
- II–B–2–c Special Laboratory Design
- II–B–3 P3 Level
- II–B–3–a Laboratory Practices
- II–B–3–b Containment Equipment
- II–B–3–c Special Laboratory Design
- II–B–4 P4 Level
- II–B–4–a Laboratory Practices
- II–B–4–b Containment Equipment
- II–B–4–c Special Laboratory Design
- II–C Shipment
- II–D Biological Containment
- II–D–1 Levels of Biological Containment
- II–D–1–a HV1
- II–D–1–b HV2
- II–D–1–c HV3
- II–D–2 Certification of Host-Vector Systems
- II–D–2–a Responsibility
- II–D–2–b Data To Be Submitted for Certification
- II–D–3 Distribution of Certified Host-Vectors

III. Containment Guidelines for Covered Experiments
- III–O Classification of Experiments Using the *E. coli* K–12 Host-Vector Systems
- III–O–1 Experiments Involving Class 3 Organisms
- III–A Classification of Experiments Using Certain HV1 and HV2 Systems
- III–A–1 Shotgun Experiments
- III–A–1a Eukaryotic DNA Recombinants
- III–A–1–b Prokaryotic DNA Recombinants
- III–A–2–a Viruses of Eukaryotes
- III–A–2–b Eukaryotic Organelle DNAs
- III–A–2–c Prokaryotic Plasmid and Phage DNAs
- III–A–3 Lowering of Containment Levels for Characterized or Purified DNA Preparations and Clones
- III–A–3–a Purified DNA Other than Plasmids, Bacteriophages, and Other Viruses
- III–A–3–b Characterized Clones of DNA Recombinants
- III–B Experiments with Prokaryotic Host-Vectors Other then *E. coli* K–12
- III–B–1 HV1 and HV2 Systems
- III–B–2 Return of DNA Segments to Prokaryotic Non-HV1 Host or Origin
- III–B–3 Non-HV1 Systems
- III–C Experiments with Eukaryotic Host-Vectors
- III–C–1 Vertebrate Host-Vector Systems
- III–C–1–a Polyoma Virus
- III–C–1–b Simian Virus 40
- III–C–1–c Human Adenoviruses 2 and 5
- III–C–1–d Murine Adenovirus Strain FL
- III–C–1–e All Viral Vectors
- III–C–1–f Nonviral Vectors
- III–C–2 Invertebrate Host-Vector Systems
- III–C–2–a Insect Viral Vectors
- III–C–2–b Nonviral Vectors
- III–C–3 PLant Viral Host-Vector Systems
- III–C–4 Plant Host-Vector Systems Other than Viruses
- III–C–5 Fungal or Similar Lower Eukaryotic Host-Vector Systems
- III–C–6 Return of DNA Segments to a Higher Eukaryotic Host of Origin
- III–C–7 Transfer of Cloned DNA Segments to Eukaryotic Organisms
- III–C–7–a Transfer to Non-human Vertebrates
- III–C–7–b Transfer to Higher Plants
- III–D Complementary DNAs
- III–E Synthetic DNAs

IV. Roles and Responsibilities
- IV–A Policy
- IV–B General Applicability
- IV–C General Definitions
- IV–D Responsibilities of the Institution
- IV–D–1 (General)
- IV–D–2 Membership and Procedures of the IBC
- IV–D–3 Functions of the IBC
- IV–D–4 Biological Safety Officer
- IV–D–5 Principal Investigator
- IV–D–5–a PI—General
- IV–D–5–b Submissions by the PI to NIH
- IV–D–5–c Submissions by the PI to the IBC
- IV–D–5–d PI Responsibilities After Approval but Prior to Initiating the Research
- IV–D–5–e PI Responsibilities During the Conduct of the Approved Research
- IV–E Responsibilities of NIH
- IV–E–1 Director
- IV–E–1–a General Responsibilities of the Director, NIH
- IV–E–1–b Specific Responsibilities of the Director, NIH
- IV–E–2 Recombinant Advisory Committee
- IV–E–3 The Office of Recombinant DNA Activities
- IV–E–4 Other NIH Components
- IV–G Compliance

V. Footnotes and References

VI. Voluntary Compliance
- VI–A Basic Policy
- VI–B IBC Approval
- VI–D Certification of Host-Vector Systems
- VI–E Requests for Exceptions, Exemptions, Approvals
- VI–F Protection of Proprietary Data

Appendix A Exemptions Under I–E–4

Appendix B Classification of Microorganisms on the Basis of Hazard

Appendix C Exemptions Under I–E–5

Appendix D HV1 and HV2 Host-Vector Systems Assigned Containment Levels as Specified in the Subsections of Section III–A

Appendix E Actions Taken Under the Guidelines

Appendix F Certified HV2 Host-Vector Systems

I. Scope of the Guidelines

I–A. *Purpose.* The purpose of these Guidelines is to specify practices for constructing and handling (i) recombinant DNA molecules and (ii) organisms and viruses containing recombinant DNA molecules.

I–B. *Definition of Recombinant DNA Molecules.* In the context of these Guidelines, recombinant DNA molecules are defined as either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules that result from the republication of those described in (i) above.

I–C. *General Applicability.* See Section IV–B.

I–D. *Prohibitions.* The following experiments are not to be initiated at the present time:

I–D–1. Formation of recombinant DNAs derived from the pathogenic organisms classified (*1*) as Class 4 or 5 (*2*) or from cells known (*2A*) to be infected with such agents, regardless of the host-vector system used.

I–D–2. Deliberate formation of recombinant DNAs containing genes for the biosynthesis of toxins potent for vertebrates (*2A*) (e.g., botulinum or diphtheria toxins; venoms from insects, snakes, etc.).

I–D–3. (Deleted).

I–D–4. Deliberate release into the environment of any organism containing recombinant DNA.

I–D–5. Deliberate transfer of a drug resistance trait to microorganisms that are not known to acquire it naturally, if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture. (*2A*)

I–D–6. Large-scale experiments (e.g., more than 10 liters of culture) with organisms containing recombinant DNAs, unless the recombinant DNAs are rigorously characterized and the absence of harmful sequences established (*a*). (See Section IV–E–1–b–(3)–(d).)

I–D (1–6). Experiments in Categories I–D–1 to I–D–6 may be expected (*4*) from the prohibitions (and will at that time be assigned appropriate levels of physical and biological containment) provided that these experiments are expressly

approved by the Director, National Institutes of Health (NIH), with advice of the Recombinant DNA Advisory Committee (RAC), after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(e).)

Experiments in Categories I–D–1, I–D–2, I–D–5, and experiments involving "wild type" host-vector systems are excepted from the prohibitions, provided that these experiments are designed for risk-assessment purposes and are conducted within the NIH high-containment facilities located in Building 41–T on the Bethesda campus and in Building 550 located at the Frederick Cancer Research Center. The selection of laboratory practices and containment equipment for such experiments shall be approved by the Office of Recombinant DNA Activities (ORDA) following consultation with the RAC Risk Assessment Subcommittee and the NIH Biosafety Committee. ORDA shall inform RAC members of the proposed risk-assessment projects at the same time it seeks consultation from the RAC Risk Assessment Subcommittee and the NIH Biosafety Committee. If a major biohazard is determined, the clones will be destroyed after the completion of the experiment rather than retaining them in the high containment facility. Other clones that are non-hazardous or not of major hazard will be retained in the high containment.

I–E. *Exemptions.* It must be emphasized that the following exemptions (*4*) are not meant to apply to experiments described in the Sections I–D–1 to I–D–5 as being prohibited. In addition, any recombinant DNA molecules involving DNA from Class 3 organisms (*1*) or cells known to be infected with these agents, or any recombinant DNA molecules which increase the virulence and host-range of a plant pathogen beyond that which occurs by natural genetic exchange, are not exempt unless specifically so designated by NIH under Section I–E–5.

The following recombinant DNA molecules are exempt from these Guidelines, and no registration with NIH is necessary:

I–E–1. Those that are not in organisms or viruses. (*5*)

I–E–2. Those that consist entriely of DNA segments from a single nonchromosomal or viral DNA source, though one or more of the segments may be a synthetic equivalent.

I–E–3. Those that consist entirely of DNA from a prokaryotic host, including its indigenous plasmids or viruses, when propagated only in that host (or a closely related strain of the same species) or when transferred to another host by well established physiological means; also those that consist entirely of DNA from a eukaryotic host, including its chloroplasts, mitochondria, or plasmids (but excluding viruses), when propagated only in that host (or a closely related strain of the same species).

I–E–4. Ceratin specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

I–E–5. Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the RAC, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix C. An updated list may be obtained from the Office of Recombinant DNA Activities; National Institutes of Health, Bethesda, Maryland, 20205.

I–F. *General Definitions.* See section IV–C.

II. Containment

Effective biological safety programs have been operative in a variety of laboratories for many years. Considerable information, therefore, already exists for the design of physical containment facilities and the selection of laboratory procedures applicable to organisms carrying recombinant DNAs. (*6–19*) The existing programs rely upon mechanisms that, for convenience, can be divided into two categories: (i) a set of standard practices that are generally used in microbiological laboratories, and (ii) special procedures, equipment, and laboratory installations that provide physical barriers which are applied in varying degrees according to the estimated biohazard.

Experiments on recombinant DNAs, by their very nature, lend themselves to a third containment mechanism—namely, the application of highly specific biological barriers. In fact, natural barriers do exist which limit either (i) the infectivity of a *vector*, or *vehicle*, (plasmid or virus) for specific hosts or (ii) its dissemination and survival in the environment. The vectors that provide the means for replication of the recombinant DNAs and/or the host cells in which they replicate can be genetically designed to decrease by many orders of magnitude the probability of dissemination of recombinant DNAs outside the laboratory.

As these three means of containment are complementary, different levels of containment appropriate for experiments with different recombinants can be established by applying various combinations of the physical and biological barriers along with a constant use of the standard practices. We consider these categories of containment separately here in order that such combinations can be conveniently expressed in the Guidelines.

In constructing these Guidelines, it was necessary to define boundary conditions for the different levels of physical and biological containment and for the classes of experiments to which they apply. We recognize that these definitions do not take into account all existing and anticipated information on special procedures that will allow particular experiments to be carried out under different conditions than indicated here without affecting risk. Indeed, we urge that individual investigators devise simple and more effective containment procedures and that investigators and institutuional biosafety committees recommend changes in the Guidelines to permit their use.

II–A. *Standard Practices and Training.* The first principle of containment is a strict adherence to good microbiological practices. (*6–15*) Consequently, all personnel directly or indirectly involved in experiments on recombinant DNAs must receive adequate instruction. (see Sections IV–D–1–g, IV–D–5–d and IV–D–8–b.). This shall, as a minimum, include instructions in aseptic techniques and in the biology of the organisms used in the experiments, so that the potential biohazards can be understood and appreciated.

Any research group working with agents with a known or potential biohazard shall have an emergency plan which describes the procedures to be followed if an accident contaminates personnel or the environment. The principal investigator must ensure that everyone in the laboratory is familiar with both the potential hazards of the

work and the emergency plan. (See Sections IV–D–5–e and IV–D–3–d.) If a research group is working with a known pathogen where there is an effective vaccine it should be made available to all workers. Where serological monitoring is clearly appropriate it shall be provide. (See Sections IV–D–1–h and IV–D–8–c.)

II–B. *Physical Containment Levels.* The objective of physical containment is to confine organisms containing recombinant DNA molecules, and thus to reduce the potential for exposure of the laboratory worker, persons outside of the laboratory, and the environment to organisms containing recombinant DNA molecules. Physical containment is achieved through the use of laboratory practices, containment equipment, and special laboratory design. Emphasis is placed on primary means of physical containment which are provided by laboratory practices and containment equipment. Special laboratory design provides a secondary means of protection against the accidental release of organisms outside the laboratory or to the environment. Special laboratory design is used primarily in facilities in which experiments of moderate to high potential hazards are performed.

Combinations of laboratory practices, containment equipment, and special laboratory design can be made to achieve different levels of physical containment. Four levels of physical containment, which are designated as P1, P2, P3, and P4, are described. It should be emphasized that the descriptions and assignments of physical containment detailed below are based on existing approaches to containment of pathogenic organisms. For example, the "Classification of Etiologic Agents on the Basis of Hazard,"(*7*) prepared by the Centers for Disease Control, describes four general levels which roughly correspond to our descriptions for P1, P2, P3, and P4; and the National Cancer Institute describes three levels for research on oncogenic viruses which roughly correspond to our P2, P3, and P4 levels.(*8*)

It is recognized that several different combinations of laboratory practices, containment equipment, and special laboratory design may be appropriate for containment of specific research activities. The Guidelines, therefore, allow alternative selections of primary containment equipment within facilities that have been designed to provide P3 and P4 levels of physical containment. The selection of alternative methods of primary containment is dependent, however, on the level of biological containment provided by the host-vector system used in the experiment. Consideration will also be given by the Director, NIH, with the advice of the Recombinant DNA Advisory Committee to other combinations which achieve an equivalent level of containment. (See Section IV–E–1–b–(2)–(b).) Additional material on physical containment for plant host-vector systems is found in Sections III–C–3 and III–C–4.

II–B–1. *P1 Level.*

II–B–1–a. *Laboratory Practices.*

II–B–1–a–(1). Laboratory doors shall be kept closed while experiments are in progress.

II–B–1–a–(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

II–B–1–a–(3). All biological wastes shall be decontaminated before disposal. Other contaminated materials, such as glassware, animal cages, and laboratory equipment, shall be decomtaminated before washing, reuse, or disposal.

II–B–1–a–(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II–B–1–a–(5). Eating, drinking, smoking, and storage of foods are not permitted in the laboratory area in which recombinant DNA materials are handled.

II–B–1–a–(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II–B–1–a–(7). Care shall be taken in the conduct of all procedures to minimize the creation of aerosols.

II–B–1–a–(8). Contaminated materials that are to be decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II–B–1–a–(9). An insect and rodent control program shall be instituted.

II–B–1–a–(10). The use of laboratory gowns, coats, or uniforms is discretionary with the laboratory supervisor.

II–B–1–a–(11). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II–B–1–a–(12). The laboratory shall be kept neat and clean.

II–B–1–b. *Containment Equipment.* Special containment equipment is not required at the P1 level.

II–B–1–c. *Special Laboratory design.* Special laboratory design is not required at the P1 level.

II–B–2. *P2 Level.*

II–B–2–a. *Laboratory Practices.*

II–B–2–a–(1). Laboratory doors shall be kept closed while experiments are in progress.

II–B–2–a–(2). Work surfaces shall be decontaminated daily, and immediately following spills of organisms containing recombinant DNA molecules.

II–B–2–a–(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a means demonstrated to be effective before washing, reuse, or disposal.

II–B–2–a–(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II–B–2–a–(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II–B–2–a–(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II–B–2–a–(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II–B–2–a–(8). Contaminated materials that are to be steam sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II–B–2–a–(9). Only persons who have been advised of the nature of the research being conducted shall enter the laboratory.

II–B–2–a–(10). The universal biohazard sign shall be posted on all laboratory access doors when experiments requiring P2 containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II–B–2–a–(11). An insect and rodent control program shall be instituted.

II–B–2–a–(12). The use of laboratory gowns, coats, or uniforms is required. Laboratory clothing shall not be worn to the lunch room or outside of the building in which the laboratory is located.

II–B–2–a–(13). Animals not related to the experiment shall not be permitted in the laboratory.

II–B–2–a–(14). Use of the hypodermic needle and syringe shall be avoided when alternative methods are available.

II–B–2–a–(15). The laboratory shall be kept neat and clean.

II–B–2–a–(16). Experiments of lesser biohazard potential can be carried out concurrently in carefully demarcated areas of the same laboratory.

II–B–2–b. *Containment Equipment.* Biological safety cabinets (20) shall be used to contain aerosol-producing equipment, such as blenders, lyophilizers, sonicators, and centrifuges, when used to process organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol. For example, a centrifuge may be operated in the open if a sealed head or safety centrifuge cups are used.

II–B–2–c. *Special Laboratory Design.* An autoclave for sterilization of wastes and contaminated materials shall be available in the same building in which organisms containing recombinant DNA molecules are used.

II–B–3. *P3 Level.*

II–B–3–a. *Laboratory Practices.*

II–B–3–a–(1). Laboratory doors shall be kept closed while experiments are in progress.

II–B–3–a–(2). Work surfaces shall be decontaminated following the completion of the experimental activity, and immediately following spills of organisms containing recombinant DNA molecules.

II–B–3–a–(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials, such as glassware, animal cages, laboratory equipment, and radioactive wastes, shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II–B–3–a–(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II–B–3–a–(5). Eating, drinking, smoking, and storage of food are not permitted in the laboratory area in which recombinant DNA materials are handled.

II–B–3–a–(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II–B–3–a–(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II–B–3–a–(8). Contaminated materials that are to be steam-sterilized (autoclaved) or decontaminated at a site away from the laboratory shall be placed in a durable leak-proof container, which is closed before removal from the laboratory.

II–B–3–a–(9). Entry into the laboratory shall be through a controlled access area. Only persons who have been advised of the nature of the research being conducted shall enter the controlled access area. Only persons required on the basis of program or support needs shall be authorized to enter the laboratory. Such persons shall be advised of the nature of the research being conducted before entry, and shall comply with all required entry and exit procedures.

II–B–3–a–(10). Persons under 16 years of age shall not enter the laboratory.

II–B–3–a–(11). The universal biohazard sign shall be posted on the controlled access area door and on all laboratory doors when experiments requiring P3-level containment are in progress. Freezers and refrigerators or other units used to store organisms containing recombinant DNA molecules shall also be posted with the universal biohazard sign.

II–B–3–a–(12). An insect and rodent control program shall be instituted.

II–B–3–a–(13). Laboratory clothing that protects street clothing (e.g., long-sleeve solid-front or wrap-around gowns, no-button or slipover jackets) shall be worn in the laboratory. Front-button laboratory coats are unsuitable. Laboratory clothing shall not be worn outside the laboratory and shall be decontaminated before it is sent to the laundry.

II–B–3–a–(14). Raincoats, overcoats, topcoats, coats, hats, caps, and such street outer-wear shall not be kept in the laboratory.

II–B–3–a–(15). Gloves shall be worn when handling materials requiring P3 containment. They shall be removed aseptically immediately after the handling procedure and decontaminated.

II–B–3–a–(16). Animals and plants not related to the experiment shall not be permitted in the laboratory.

II–B–3–a–(17). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II–B–3–a–(18). Use of hypodermic needle and syringe shall be avoided when alternative methods are available.

II–B–3–a–(19). The laboratory shall be kept neat and clean.

II–B–3–a–(20). If experiments involving other organisms which require lower levels of containment are to be conducted in the same laboratory concurrently with experiments requiring P3-level physical containment, they shall be conducted in accordance with all P3-level laboratory practices.

II–B–3–b. *Containment Equipment.*

II–B–3–b–(1) Biological safety cabinets (*20*) shall be used for all equipment and manipulations that produce aerosols—e.g., pipetting, dilutions, transfer operations, plating, flaming, grinding, blending, drying, sonicating, shaking, centrifuging—where these procedures involve organisms containing recombinant DNA molecules, except where equipment design provides for containment of the potential aerosol.

II–B–3–b–(2). Laboratory animals held in a P3 area shall be housed in partial-containment caging systems, such as Horsfall units (19A), open cages placed in ventilated enclosures, solid-wall and bottom cages covered by filter bonnets, or solid-wall and -bottom cages placed on holding racks equipped with ultraviolet radiation lamps and reflectors. (Note: Conventional caging systems may be used, provided that all personnel wear appropriate personal protective devices. These shall include, at a minimum, wrap-around gowns, head covers, gloves, shoe covers, and respirators. All personnel shall shower on exit from areas where these devices are required.)

II–B–3–b–(3). *Alternative Selection of Containment Equipment.*

Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified in Part III can be conducted in the P3 laboratory using containment equipment specified for the P2 level of physical containment. Experimental procedures involving a host-vector system that provides a one-step lower level of biological containment than that specified in Part III can be conducted in the P3 laboratory using containment equipment specified for the P4 level of physical containment. Alternative combinations of containment safeguards are shown in Table I.

Table I.—*Combinations of Containment Safeguards*

Classification of experiment According to guidelines		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological [1] containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	Biological containment
P3	HV3	P3	P3	P3	HV3
P3	HV3	P3	P3	P4	HV2
P3	HV2	P3	P3	P3	HV2
P3	HV2	P3	P3	P2	HV3

Table I.—*Combinations of Containment Safeguards*

Classification of experiment According to guidelines		Alternate combinations of physical and biological containment			
		Physical containment			
Physical containment	Biological [1] containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	Biological containment
P3	HV2	P3	P3	P4	HV1
P3	HV1	P3	P3	P3	HV1
P3	HV1	P3	P3	P2	HV2

[1] See Section II-D for decription of biological containment.

II–B–3–c. *Special Laboratory Design.*

II–B–3–c–(1). The laboratory shall be separated by a controlled access area from areas that are open to unrestricted traffic flow. A controlled access area is an anteroom, a change room, an air lock or any other double-door arrangement that separates the laboratory from areas open to unrestricted traffic flow.

II–B–3–c–(2). The surfaces of walls, floors, and ceilings shall be readily cleanable. Penetrations through these surfaces shall be sealed or capable of being sealed to facilitate space decontamination.

II–B–3–c–(3). A foot-, elbow-, or automatically-operated hand-washing facility shall be provided near each primary laboratory exit area.

II–B–3–c–(4). Windows in the laboratory shall be sealed.

II–B–3–c–(5). An autoclave for sterilization of wastes and contaminated materials shall be available in the same building (and preferably within the controlled laboratory area) in which organisms containing recombinant DNA molecules are used.

II–B–3–c–(6). The laboratory shall have a ventilation system that is capable of controlling air movement. The movement of air shall be from areas of lower contamination potential to areas of higher contamination potential (i.e., from the controlled access area to the laboratory area). If the ventilation system provides positive pressure supply air, the system shall operate in a manner that prevents the reversal of the direction of air movement or shall be equipped with an alarm that would be actuated in the event that reversal in the direction of air movement were to occur. The exhaust air from the laboratory area shall not be recirculated to other areas of the building unless the exhaust air is filtered by HEPA filters or equivalent. The exhaust air from the laboratory area can be discharged to the outdoors without filtration or other means for effectively reducing an accidental aerosol burden provided that it can be dispersed clear of occupied buildings and air intakes.

II–B–3–c–(7). The treated exhaust-air from Class I and Class II biological safety cabinets [*20*]may be discharged either to the laboratory or to the outdoors. The treated exhaust-air from a Class III cabinet shall be discharged directly to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through a building exhaust air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinet and the building ventilation system.

II–B–4. *P4 Level.*

II–B–4–a. *Laboratory Practices.*

II–B–4–a–(1). Laboratory doors shall be kept closed while experiments are in progress.

II–B–4–a–(2). Work surfaces shall be decontaminated following the completion of the experimental activity and immediately following spills of organisms containing recombinant DNA molecules.

II–B–4–a–(3). All laboratory wastes shall be steam-sterilized (autoclaved) before disposal. Other contaminated materials such as glassware, animal cages, laboratory equipment, and radioactive wastes shall be decontaminated by a method demonstrated to be effective before washing, reuse, or disposal.

II–B–4–a–(4). Mechanical pipetting devices shall be used; pipetting by mouth is prohibited.

II–B–4–a–(5). Eating, drinking, smoking, and storage of food are not permitted in the P4 facility.

II–B–4–a–(6). Persons shall wash their hands after handling organisms containing recombinant DNA molecules and when they leave the laboratory.

II–B–4–a–(7). Care shall be exercised to minimize the creation of aerosols. For example, manipulations such as inserting a hot inoculating loop or needle into a culture, flaming an inoculation loop or needle so that it splatters, and forceful ejection of fluids from pipettes or syringes shall be avoided.

II–B–4–a–(8). Biological materials to be removed from the P4 facility in a viable or intact state shall be transferred to a nonbreakable sealed container, which is then removed from the P4 facility through a pass-through disinfectant dunk tank or fumigation chamber.

II–B–4–a–(9). No materials, except for biological materials that are to remain in a viable or intact state, shall be removed from the P4 facility unless they have been steam-sterilized (autoclaved) or decontaminated by a means demonstrated to be effective as they pass out of the P4 facility. All wastes and other materials as well as equipment not damaged by high temperature or steam shall be steam sterilized in the double-door autoclave of the P4 facility. Other materials which may be damaged by temperature or steam shall be removed from the P4 facility through a pass-through fumigation chamber.

II–B–4–a–(10). Materials within the Class III cabinets shall be removed from the cabinet system only after being steam-sterilized in an attached double-door autoclave or after being contained in a nonbreakable sealed container, which is then passed through a disinfectant dunk tank or a fumigation chamber.

II–B–4–a–(11). Only persons whose entry into the P4 facility is required to meet program or support needs shall be authorized to enter. Before entering, such persons shall be advised of the nature of the research being conducted and shall be instructed as to the appropriate safeguards to ensure their safety. They shall comply with instructions and all other required procedures.

II–B–4–a–(12). Persons under 18 years of age shall not enter the P4 facility.

II–B–4–a–(13). Personnel shall enter into and exit from the P4 facility only through the clothing change and shower rooms. Personnel shall shower at each egress from the P4 facility. Air locks shall not be used for personnel entry or exit except for emergencies.

II–B–4–a–(14). Street clothing shall be removed in the outer side of the clothing-change area and kept there. Complete laboratory clothing, including undergarments, head cover, shoes, and either pants and shirts or jumpsuits, shall be used by all persons who enter the P4 facility. Upon exit, personnel shall store this clothing in lockers provided for this purpose or discard it into collection hampers before entering the shower area.

II–B–4–a–(15). the universal biohazard sign is required on the P4 facility access doors and on all interior doors to individual laboratory rooms where experiments are conducted. The sign shall also be posted on freezers, refrigerators, or other units used to store organisms containing recombinant DNA molecules.

II–B–4–a–(16). An insect and rodent control program shall be instituted.

II–B–4–a–(17). Animals and plants not related to the experiment shall not be

permitted in the laboratory in which the experiment is being conducted.

II–B–4–a–(18). Vacuum outlets shall be protected by filter and liquid disinfectant traps.

II–B–4–a–(19). Use of the hypodermic needle and syringe shall be avoided when alternate methods are available.

II–B–4–a–(20). The laboratory shall be kept neat and clean.

II–B–4–a–(21) If experiments involving other organisms which require lower levels of containment are to be conducted in the P4 facility concurrently with experiments requiring P4–level containment, they shall be conducted in accordance with all P4–level laboratory practices specified in this section.

II–B–4–b. *Containment Equipment.*

II–B–4–b–(1). Experimental procedures involvong organisms that require P4-level physical containment shall be conducted either in (i) a Class III cabinet system or in (ii) Class I or Class II cabinets that are located in a specially designed area in which all personnel are required to wear one-piece positive-pressure isolation suits.

II–B–4–b–(2). Laboratory animals involved in experiments requiring P4-level physical containment shall be housed either in cages contained in Class III cabinets or in partial containment caging systems (such as Horsfall units [19A], open cages placed in ventilated enclosures, or solid-wall and -bottom cages covered by filter bonnets, or solid-well and -bottom cages placed on holding racks equipped with ultraviolet irradiation lamps and reflectors) that are located in a specially designed area in which all personnel are required to wear one-piece positive-pressure suits.

II–B–4–b–(3). *Alternative Selection of Containment Equipment.* Experimental procedures involving a host-vector system that provides a one-step higher level of biological containment than that specified in Part III can be conducted in the P4 facility using containment equipment requirements specified for the P3 level of physical containment. Alternative combinations of containment safeguards are shown in Table II

Table II.—*Combinations of Containment Safeguards*

Classification of experiment according to guidelines		Alternate combinations of physical and biological containment			
		Physical containment			Biological containment
Physical containment	Biological [1] containment	Laboratory design specified for—	Laboratory practices specified for—	Containment equipment specified for—	
P4	HV1	P4	P4	P4	HV1
P4	HV1	P4	[2]P4	P3	HV2

[1] See Section II–D for description of biological containment.
[2] In this case gloves shall be worn, in addition to the clothing requirements specified in II–B–4–a–(14).

II–B–4–c. *Special Laboratory Design.*

II–B–4–c–(1). The laboratory shall be located in a restricted-access facility which is either a separate building or a clearly demarcated and isolated zone within a building. Clothing-change areas and shower rooms shall be provided for personnel entry and egress. These rooms shall be arranged so that personnel leave through the shower area to the change room. A double-door ventilated vestibule or ultraviolet air lock shall be provided for passage of materials, supplies, and equipment which are not brought into the P4 facility through the change room area.

II–B–4–c–(2). Walls, floors, and ceilings of the P4 facility are constructed to form an internal shell which readily allows vapor-phase decontamination and is animal- and insect-proof. All penetrations through these structures and surfaces ar sealed. (The integrity of the walls, floors, ceilings, and penetration seals should ensure adequate containment of a vapor-phase decontaminant under static pressure conditions. This requirement does not imply that these surfaces must be airtight.)

II–B–4–c–(3). A foot-, elbow-, or automatically-operated handwashing facility shall be provided near the door within each laboratory in which experiments involving recombinant DNA are conducted in openface biological safety cabinets.

II–B–4–c–(4). Central vacuum systems are permitted. The system, if provided, shall not serve areas outside the P4 facility. The vacuum system shall include in-line HEPA filters near each use point or service cock. The filters shall be installed so as to permit in-place decontamination and replacement. Water supply, liquid and gaseous services provided to the P4 facility shall be protected by devices that prevent backflow.)

II–B–4–c–(5). Drinking water fountains shall not be installed in laboratory or animal rooms of the P4 facility. Foot-operated water fountains are permitted in the corridors of the P4 facility. The water service provided to such fountains shall be protected from the water services to the laboratory areas of the P4 facility.

II–B–4–c–(6). Laboratory doors shall be self-closing.

II–B–4–c–(7). A double-door autoclave shall be provided for sterilization of material passing out of the P4 facility. The autoclave doors shall be interlocked so that both doors will not be open at the same time.

II–B–4–c–(8). A pass-through dunk tank or fumigation chamber shall be provided for removal from the P4 facility of material and equipment that cannot be heat-sterilized.

II–B–4–c–(9). All liquid effluents from the P4 facility shall be collected and decontaminated before disposal. Liquid effluents from biological safety cabinets and laboratory sinks shall be sterilized by heat. Liquid effluents from the shower and hand washing facilities may be activated by chemical treatment. HEPA filters shall be installed in all vents from effluent drains.

II–B–4–c–(10). An individual supply and exhaust-air ventilation system shall be provided. The system shall maintain pressure differentials and directional air flow as required to ensure inflow from areas outside the facility toward areas of highest potential risk within the facility. The system shall be designed to prevent the reversal of air flow. The system shall sound an alarm in the event of system malfunction.

II–B–4–c–(11). Air within individual laboratories of the P4 facility may be recirculated if HEPA filtered.

II–B–4–c–(12). The exhaust air from the P4 facility shall be HEPA filtered and discharged to the outdoors so that it is dispersed clear of occupied buildings and air intakes. The filter chambers shall be designed to allow *in situ* decontamination before removal and to facilitate certification testing after replacement.

II–B–4–c–(13). The treated exhaust-air from Class I and Class II biological safety cabinets(*20*) may be discharged directly to the laboratory room environment or to the outdoors. The treated exhaust-air from Class III cabinets shall be discharged to the outdoors. If the treated exhaust-air from these cabinets is to be discharged to the outdoors through the P4 facility exhaust

air system, it shall be connected to this system so as to avoid any interference with the air balance of the cabinets of the facility exhaust air system.

II–B–4–c–(14). As noted in Section II–B–4–b–(1), the P4 facility may contain specially designed areas in which all personnel are required to wear one-piece positive-pressure isolation suits. Such areas shall be airtight. The exhaust-air from the suit area shall be filtered by two sets of HEPA filters installed in series, and a duplicate filtration unit and exhaust fan shall be provided. The air pressure within the suit area shall be less than that in any adjacent area. An emergency lighting system, communication systems, and power source shall be provided. A double-door autoclave shall be provided for sterilization of all waste materials to be removed from the suit area.

Personnel who enter this area shall wear a one-piece positive-pressure suit that is ventilated by a life-support system. The life-support system shall be provided with alarms and emergency backup air. Entry to this area is through an airlock fitted with airtight doors. A chemical shower area shall be provided to decontaminate the surfaces of the suit before removal.

II–C. *Shipment.* Recombinant DNA molecules contained in an organism or virus shall be shipped only as an etiologic agent under requirements of the U.S. Public Health Service, and the U.S. Department of Transportation (§ 72.25, Part 72, Title 42, and §§ 173.386–.388, Part 173, Title 49, U.S. Code of Federal Regulations (CFR)) as specified below:

II–C–1. Recombinant DNA molecules contained in an organism or virus requiring P1, P2, or P3 physical containment, when offered for transportation or transported, are subject to all requirements of § 72.25(c)(1—(5), Part 72, Title 42 CFR, and §§ 173.386–.388, Part 173, Title 49 CFR.

II–C–2. Recombinant DNA molecules contained in an organism or virus requiring P4 physical containment, when offered for transportation or transported, are subject to the requirements listed above under II–C–1 and are also subject to § 72.25(c)(6), Part 72, Title 42 CFR.

II–C–3. Additional information on packaging and shipment is given in the "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research."

II–D. *Biological Containment.*

II–D–1. *Levels of Biological Containment.* In consideration of biological containment, the vector (plasmid, organelle, or virus) for the recombinant DNA and the host (bacterial, plant, or animal cell) in which the vector is propagated in the laboratory will be considered together. Any combination of vector and host which is to provide biological containment must be chosen or constructed so that the following types of "escape" are minimized: (i) survival of the vector in its host outside the laboratory and (ii) transmission of the vector from the propagation host to other nonlaboratory hosts.

The following levels of biological containment (HV, or *H*ost-*V*ector, systems) for prokaryotes will be established; specific criteria will depend on the organisms to be used. Eukaryotic host-vector systems are considered in Part III.

II–D–1–a. *HV1.* A host-vector system which provides a moderate level of containment. *Specific systems:*

II–D–1–a–(1). *EK1.* The host is always *E. coli* K–12 or a derivative thereof, and the vectors include nonconjugative plasmids (e.g., pSC101, ColE1, or derivatives thereof [*21–27*]) and variants of bacteriophage, such as lambda (*28–33*). The *E. coli* K–12 hosts shall not contain conjugation-proficient plasmids, whether autonomous or integrated, or generalized transducing phages, except as specified in Section III–0.

II–D–1–a–(2). *Other Prokaryotes.* Hosts and vectors shall be, at a minimum, comparable in containment to *E. coli* K–12 with a non conjugative plasmid or bacteriophage vector. The data to be considered and a mechanism for approval of such HV1 systems are described below (Section II—D–2).

II–D–1–b. *HV2.* These are host-vector systems shown to provide a high level of biological containment as demonstrated by data from suitable tests performed in the laboratory. Escape of the recombinant DNA either via survival of the organisms or via transmission of recombinant DNA to other organisms should be less than $1/10^8$ under specified conditions. *Specific systems:*

II–D–1–b–(1). For EK2 host-vector systems in which the vector is a plasmid, no more than one in 10^8 host cells should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment, either by survival of the original host or as a consequence of transmission of the cloned DNA fragment.

II–D–1–b–(2). For EK2 host-vector systems in which the vector is a phage, no more than one in 10^8 phage particles should be able to perpetuate a cloned DNA fragment under the specified nonpermissive laboratory conditions designed to represent the natural environment either (i) as a prophage (in the inserted or plasmid form) in the laboratory host used for phage propagation or (ii) by surviving in natural environments and transferring a cloned DNA fragment to other hosts (or their resident prophages).

II–D–1–c. *HV3.* These are host-vector systems in which:

II–D–1–c–(1). All HV2 criteria are met.

II–D–1–c–(2). The vector is dependent on its propagation host or is highly defective in mobilizability. Reversion to host-independence must be less than $1/10^8$ per vector genome per generation.

II–D–1–c–(3). No markers conferring resistance to antibiotics commonly used clinically or in agriculture are carried by the vector, unless expression of such markers is dependent on the propagating host or on unique laboratory-controlled conditions or is blocked by the inserted DNA.

II–D–1–c–(4). The specified containment shown by laboratory tests has been independently confirmed by specified tests in animals, including primates, and in other relevant environments.

II–D–1–c–(5). The relevant genotypic and phenotypic traits have been independently confirmed.

II–D–2. *Certification of Host-Vector Systems.*

II–D–2–a. *Responsibility.* HV1 systems other than *E. coli* K–12, and HV2 and HV3 host-vector systems, may not be designated as such until they have been certified by the director, NIH. Application for certification of a host-vector system is made by written application to the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

Host-vector systems that are proposed for certification will be reviewed by the National Institutes of Health (NIH) Recombinant DNA Advisory Committee (RAC). (See Section IV–E–1–b–(1)–(c).) This will first involve review of the data on construction, properties, and testing of the proposed host-vector system by a Working Group composed of one or more members of the RAC and other persons chosen because of their expertise in evaluating such data. The Committee will then evaluate the report of the Working Group and any other available information at a regular meeting. The Director, NIH, is responsible for certification after receiving the advice of the RAC. Minor modifications of existing certified host-vector systems, where the modifications are of minimal or no consequence to the properties relevant to containment may be certified by the Director, NIH,

without review by the RAC. (See Section IV–E–1–b–(3)–(f).)

When new host-vector systems are certified, notice of the certification will be sent by the Office of Recombinant DNA Activities (ORDA) to the applicant and to all Institutional Biosafety Committees (IBCs) and will be published in the *Recombinant DNA Technical Bulletin.* Copies of a list of all currently certified host-vector systems may be obtained from ORDA at any time.

The Director, NIH, may at any time rescind the certification of any host-vector system. (See Section IV–E–1–b–(3)–(i).) If certification of a host-vector system is rescinded, NIH will instruct investigators to transfer cloned DNA into a different system, or use the clones at a higher physical containment level unless NIH determines that the already constructed clones incorporate adequate biological containment.

Certification of a given system does not extend to modifications of either the host or vector component of that system. Such modified systems must be independently certified by the Director, NIH. If modifications are minor, it may only be necessary for the investigator to submit data showing that the modifications have either improved or not impaired the major phenotypic traits on which the containment of the system depends. Substantial modifications of a certified system require the submission of complete testing data.

II–D–2–b. *Data To Be Submitted for Certification.*

II–D–2–b–(1). *HV1 Systems Other than E. Coli K–12.* The following types of data shall be submitted, modified as appropriate for the particular system under consideration: (i) A description of the organism and vector; the strain's natural habitat and growth requirements; its physiological properties, particularly those related to its reproduction and survival and the mechanisms by which it exchanges genetic information; the range of organisms with which this organism normally exchanges genetic information and what sort of information is exchanged; and any relevant information on its pathogenicity or toxicity. (ii) A description of the history of the particular strains and vectors to be used, including data on any mutations which render this organism less able to survive or transmit genetic information. (iii) A general description of the range of experiments contemplated, with emphasis on the need for developing such an HV1 system.

II–D–2–b–(2). *HV2 Systems.* Investigators planning to request HV2 certification for host-vector systems can obtain instructions from ORDA concerning data to be submitted (*33A, 33B*). In general, the following types of data are required: (i) Description of construction steps, with indication of source, properties, and manner of introduction of genetic traits. (ii) Quantitative data on the stability of genetic traits that contribute to the containment of the system. (iii) Data on the survival of the host-vector system under nonpermissive laboratory conditions designed to represent the relevant natural environment. (iv) Data on transmissibility of the vector and/or a cloned DNA fragment under both permissive and nonpermissive conditions. (v) Data on all other properties of the system which affect containment and utility, including information on yields of phage or plasmid molecules, ease of DNA isolation, and ease of transfection or transformation. (vi) In some cases, the investigator may be asked to submit data on survival and vector transmissibility from experiments in which the host-vector is fed to laboratory animals (e.g., rodents). Such *in vivo* data may be required to confirm the validity of predicting *in vivo* survival on the basis of *in vitro* experiments.

Data must be submitted in writing to ORDA. Ten to twelve weeks are normally required for review and circulation of the data prior to the meeting at which such data can be considered by the RAC. Investigators are encouraged to publish their data on the construction, properties, and testing of proposed HV2 systems prior to consideration of the system by the RAC and its subcommittee. More specific instructions concerning the type of data to be submitted to NIH for proposed EK2 systems involving either plasmids or bacteriophage λ in *E. coli* K–12 are available from ORDA.

II–D–2–b–(3). *HV3 Systems.* Putative HV3 systems must, as the first step in certification, be certified as HV2 systems. Systems which meet the criteria given above under II–D–1–(c)–1, II–D–1–(c)–2, and II–D–1–(c)–3 will then be recommended for HV3 testing. Tests to evaluate various HV2 host-vector systems for HV3 certification will be performed by contractors selected by NIH. These contractors will repeat tests performed by individuals proposing the HV2 system and, in addition, will conduct more extensive tests on conditions likely to be encountered in nature. The genotypic and phenotypic traits of HV2 systems will be evaluated. Tests on survival and transmissibility in and on animals, including primates, will be performed, as well as tests on survival in certain specified natural environments.

II–D–3. *Distribution of Certified Host-Vectors.* Certified HV2 and HV3 host-vector systems (plus appropriate control strains) must be obtained from the NIH or its designees, one of whom will be the investigator who developed the system. NIH shall announce the availability of the system by publication of notices in appropriate journals.

Plasmid vectors will be provided in a suitable host strain, and phage vectors will be distributed as small-volume lysates. If NIH propagates any of the host strains or phage, a sample will be sent to the investigator who developed the system or to an appropriate contractor, prior to distribution, for verification that the material is free from contamination and unchanged in phenotypic properties.

In distributing the certified HV2 and HV3 host-vector systems, NIH or its designee will (i) send out a complete description of the system; (ii) enumerate and describe the tests to be performed by the user in order to verify important phenotypic traits; (iii) remind the user that any modification of the system necessitates independent approval of the system by the NIH; and (iv) remind the user of responsibility for notifying ORDA of any discrepancies with the reported properties or any problems in the safe use of the system.

NIH may also distribute certified HV1 host-vector systems.

III. Containment Guidelines for Covered Experiments

Part III discusses experiments covered by the Guidelines. The reader must first consult Part I, where listings are given of prohibited and exempt experiments.

Containment guidelines for permissible experiments are given in Part III. For these experiments no registration with the National Institutes of Health (NIH) is necessary. However, for these experiments, prior to their initiation, investigators must submit to their Institutional Biosafety Committee (IBC) a registration document that contains a description of (a) the source(s) of DNA, (b) the nature of the inserted DNA sequences, (c) the hosts and vectors to be used, (d) whether a deliberate attempt will be made to obtain expression of a foreign gene in the cloning vehicle and if so, what protein, and (e) the containment conditions specified by these Guidelines. This registration document must be dated and signed by the investigator and filed only with the local IBC. The IBC shall review all such proposals: IBC review prior to initiation

of the experiment is not required for most experiments described in Section III–O. Prior IBC review is required for all other experiments described in the subsections of Part III.

Changes from the levels specified in Part III for specific experiments (or the assignment of levels to experiments not explicitly considered here) may not be instituted without the express approval of the Director, NIH. (See Sections IV–E–1–b–(1)–(a), IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).)

In the classification of containment criteria for different kinds of recombinant DNAs, the stated levels of physical and biological containment are minimal for the experiments designated. The use of higher levels of biological containment (HV3<HV2<HV1) is encouraged if they are available and equally appropriate for the purposes of the experiment.

III–O. *Classification of Experiments Using E. coli K–12 and Saccharomyces cerevisiae Host-Vector Systems.* Most recombinant DNA experiments currently being done employ *E. coli* K–12 host-vector systems; others employ the *S. cerevisiae* host-vector systems. These are the systems for which we have the most experience and knowledge.

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems and prohibited (see Section I–D).

Some experiments using *E. coli* K–12 and *S. cerevisiae* host-vector systems are exempt from the Guidelines (see Section I–E).

Experiments using *E. coli* K–12 host-vector systems and DNA from Class 3 organisms [1] or from cells known to be infected with these agents will be conducted at P3 containment or at a lower level as specified by NIH (See Section IV–E–1–b–2–(e)).

Other experiments using *E. coli* K–12 or laboratory strains of *S. cerevisiae* shall use P1 physical containment and, except as specified in the last paragraph of this section, an HV1 host-vector system [i.e., for experiments using *E. coli* K–12 (a) the *E. coli* host shall not contain conjugation-proficient plasmids or generalized transducing phages, and (b) lambda or lambdoid or Ff bacteriophages or non-conjugative plasmids [49] shall be used as vectors. For experiments in *S. cerevisiae*, laboratory strains shall be used]. For these experiments review by the IBC prior to the initiation of the experiment is not required. An exception, however, which does require prior review and approval by the IBC is any experiment in which there is a deliberate attempt to have the *E. coli* K–12 efficiently express as a protein product the information carried in any gene derived from a eukaryotic organism or from any virus or viroid which infects a eukaryotic organism.

Experiments involving the insertion into *E. coli* K–12 of DNA from prokaryotes that exchange genetic information with *E. coli* by known physiological processes will be exempted from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4).

For those not on the Appendix A list but which exchange genetic information (*35*) with *E. coli*, experiments may be performed with any *E. coli* K–12 vector (e.g., conjugative plasmid). When a non-conjugative vector is used, the *E. coli* K–12 host may contain conjugation-proficient plasmids, either autonomous or integrated, or generalized transducing phages.

III–O–1. *Experiments Involving Class 3 Organisms.* Experiments involving recombinant DNA from Class 3 organisms (*1*) or from cells known to be infected with these agents may be conducted at P3 containment in *E. coli* K–12 EK1 hosts (see Section III–O). Containment levels for all other experiments with Class 3 organisms or with recombinant DNA which increases the virulence and host range of a plant pathogen beyond that which occurs by natural genetic exchange will be determined by NIH. (See Section (IV–E–1–b–2–(e)).

III–A. *Classification of Experiments Using Certain HV1 and HV2 Host-Vector Systems.* Certain HV1 and HV2 host-vector systems are assigned containment levels as specified in the subsections of this Section III–A. Those so classified as of publication of these revised Guidelines are listed in Appendix D. An updated list may be obtained from the Office of Recombinant DNA Activities, National Institutes of Health, Bethesda, Maryland 20205.

III–A–1. *Shotgun Experiments.* These experiments involve the production of recombinant DNAs between the vector and portions of the specified cellular source, preferably a partially purified fraction. Care should be taken either to preclude or eliminate contaminating microoganisms before isolating the DNA.

III–A–1–a. *Eukaryotic DNA Recombinants.*

III–A–1–a–(1). *Primates.* P2 physical containment + an HV2 host-vector or P3 + HV1.

III–A–1–a–(2). *Other Mammals.* P2 physical containment + an HV2 host-vector or P3 + HV1.

III–A–1–a–(3). *Birds.* P2 physical containment + an HV2 host-vector, or P3 + HV1.

III–A–1–a–(4). *Cold-Blooded Vertebrates.* P2 physical containment + an HV1 host-vector or P1 + HV2. If the eukaryote is known to produce a potent polypeptide toxin, (*34*) the containment shall be increased to P3 + HV2.

III–A–1–a–(5). *Other Cold-Blooded Animals and Lower Eukaryotes.* This large class of eukaryotes is divided into two groups:

III–A–1–a–(5)–a. Species that are known to produce a potent polypeptide toxin (*34*) that acts in vertebrates, or are known pathogens listed in Class 2, (*1*) or are known to carry such pathogens must use P3 physical containment + an HV2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked eukaryote genes, containment may be reduced to P3+HV1 or P2+HV2. Species that produce potent toxins that affect invertebrates or plants but not vertebrates require P2+HV2 or P3+HV1. Any species that has a demonstrated capacity for carrying particular pathogenic microorganisms is included in this group, unless the organisms used as the source of DNA have been shown not to contain those agents, in which case they may be placed in the following group. (*2A*)

III–A–1–a–(5)–(b). The remainder of the species in this class including plant pathogenic or symbiotic fungi that do not produce potent toxins: P2+HV1 or P1+HV2. However, any insect in this group must be either (i) grown under laboratory conditions for at least 10 generations prior to its use as a source of DNA, or (ii) if caught in the wild, must be shown to be free of disease-causing microorganisms or must belong to a species that does not carry microorganisms causing disease in vertebrates or plants. (*2A*) If these conditions cannot be met, experiments must be done under P3+HV1 or P2+HV2 containment.

III–A–1–a–(6). *Plants.* P2 physical containment + an HV1 host-vector, or P1+HV2. If the plant source makes a potent polypeptide toxin, (*34*) the containment must be raised to P3 physical containment + HV2 host-vector. When the potent toxin is not a polypeptide and is likely not to be the product of closely linked plant genes, containment may be reduced to P3+HV1 or P2+HV2. (*2A*)

III–A–1–b. *Prokaryotic DNA Recombinants.* P2+HV1 or P1+HV2 for experiments with phages, plasmids and DNA from nonpathogenic prokaryotes which do not produce polypeptide toxins. (*34*) P3+HV2 for experiments

with phages, plasmids and DNA from Class 2 agents. (*1*)

III–A–2–a. *Viruses of Eukaryotes* (summary given in Table III; see also exception given at asterisk at end of Appendix D).

III–A–2–a–(1). *DNA Viruses.*

III–A–2–a–(1)–(a). *Nontransforming viruses.*

III–A–2–a–(1)–(a)–(*1*). *Adeno-Associated Viruses, Minute Virus of Mice, Mouse Adenovirus (Strain FL, and Plant Viruses. (48)* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) subgenomic DNA segments, or (iii) purified cDNA copies of viral mRNA. (*37*)

III–A–2–a–(1)–(a)–(*2*). *Hepatitis B.*

III–A–2–a–(1)–(a)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for purified subgenomic DNA segments. (*38*)

III–A–2–a–(1)–(a)–(*2*)–(*b*). P2 physical containment + an HV2 host-vector, or P3 + HV1, shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(1)–(a)–(*2*)–(*c*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants derived from purified cDNA copies of viral mRNA.(*37*)

III–A–2–a–(1)–(a)–(*3*). *Other Nontransforming Member of Presently Classified Viral Families.(36)*

III–A–2–a–(1)–(a)–(*3*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for (i) DNA recombinants produced with purified subgenomic DNA(*38*) segments or (ii) purified cDNA copies of viral mRNA.(*37*)

III–A–2–a–(1)–(a)–(*3*)–(*b*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(1)–(b). *Transforming Viruses.(37A)*

III–A–2–a–(1)–(b)–(*1*). *Herpes Saimiri, Herpes Ateles, and Epstein Barr Virus.(39)*

III–A–2–a–(1)–(b)–(*1*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.(*38*)

III–A–2–a–(1)–(b)–(*1*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for (i) DNA recombinants produced with purified subgenomic DNA segments containing an entire transforming gene(*38*) or (ii) purified cDNA copies of viral mRNA.(*37*)

III–A–2–a–(1)–(b)–(*1*)–(*c*). P3 physical containment + an HV1 host-vector, or P2 + HV2, shall be used for DNA recombinants produced with the whole viral genome or with subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(1)–(b)–(*2*). *Other Transforming Members of Presently Classified Viral Families.(36)*

III–A–2–a–(1)–(b)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments(*38*)

III–A–2–a–(1)–(b)–(*2*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for (i) DNA recombinants produced with the whole viral genome, (ii) subgenomic DNA segments containing an entire transforming gene, (iii) purified cDNA copies of viral mRNA,(*37*) or (iv) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2). *DNA Transcripts of RNA Viruses.*

III–A–2–a–(2)–(a). *Retroviruses.*

III–A–2–a–(2)–(a)–(*1*). *Gibbon Ape, Woolly Monkey, Feline Leukemia and Feline Sarcoma Viruses.(39)*

III–A–2–a–(2)–(a)–(*1*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.(*38*)

III–A–2–a–(2)–(a)–(*1*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified subgenomic DNA segments.(*38*) containing an entire transforming gene.

III–A–2–a–(2)–(a)–(*1*)–(*c*). P2 physical containment + an HV2 host-vector, or P3 + HV1, shall be used for DNA recombinants produced with (i) the whole viral genome, (ii) purified cDNA copies of viral mRNA,(*37*) or (iii) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2)–(a)–(*2*). *Other Members of the Family Retroviridiae.(36)*

III–A–2–a–(2)–(a)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified nontransforming subgenomic DNA segments.(*38*)

III–A–2–a–(2)–(a)–(*2*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) subgenomic DNA segments containing an entire transforming gene, (ii) the whole viral genome, or (iii) purified cDNA copies of viral mRNA, (*37*) or (iv) subgenomic segments that have not been purified to the extent required in footnote 38.

III–A–2–a–(2)–(b). *Negative Strand RNA Viruses.* Pl physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA. (*37*)

III–A–2–a–(2)–(c). *Plus-Strand RNA Viruses.*

III–A–2–a–(2)–(c)–(*1*). *Types 1 and 2 Sabin Poliovirus Vaccine Strains and Strain 17D (Theiler) of Yellow Fever Virus.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA. (*37*)

III–A–2–a–(2)–(c)–(*2*). *Other Plus-Strand RNA Viruses Belonging to Presently Classified Viral Families. (36)*

III–A–2–a–(2)–(c)–(*2*)–(*a*). P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with purified subgenomic cDNA segments. (*38*)

Table III.—*Recommended Containment for Cloning of Viral DNA or cDNA in Certain HV1 and HV2 Systems Specified in Appendix D*

[See text for full details]

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic [38]		Genomic[1]		cDNA from viral mRNA [37]
	Non-transforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
DNA:					
Nontransforming viruses:					
AAV, MVM, mouse adeno (strain FL)	P1+HV1		P1+HV1		P1+HV1
Plant viruses	P1+HV1		P1+HV1		P1+HV1
Hepatitis B	P1+HV1[38]		P2+HV2 or P3+HV1		P2+HV1
Other	P1+HV1[38]		P1+HV1		P1+HV1
Transforming viruses:					
Herpes Saimiri, H. Ateles and EBV [39]	P1+HV1[38]	P2+HV1	P2+HV2 or P3+HV1		P2+HV1

Table III.—*Recommended Containment for Cloning of Viral DNA or cDNA in Certain HV1 and HV2 Systems Specified in Appendix D*—Continued

[See text for full details]

Virus class	Type of viral DNA segment to be cloned				
	Subgenomic [38]		Genomic[1]		cDNA from viral mRNA [37]
	Non-transforming segment	Segment containing an entire transforming gene	Nonsegmented genome	Segmented genome	
Other	P1+HV1[38]	P2+HV1	P2+HV1	.....	P2+HV1
RNA:					
Retroviruses:					
Gibbon ape, wooly monkey FeLV and FeSV [39].	P1+HV1[38]	P2+HV1	P2+HV2 or P3+HV1	.....	P2+HV2 or P3+HV1
Other	P1+HV1[38]	P2+HV1	P2+HV1	.....	P2+HV1
Negative-Strand RNA	P1+HV1	.....	P1+HV1	P1+HV1	P1+HV1
Plus-Strand RNA:					
Types 1 and 2 Sabin polio, 17D yellow fever vaccine strains.	P1+HV1	.....	P1+HV1	.....	P1+HV1
Other	P1+HV1[38]	.....	P2+HV1	.....	P2+HV1
Double-stranded RNA	P1+HV1	.....	.....	P1+HV1	P1+HV1
Plant viruses + viroids	P1+HV1	.....	P1+HV1	P1+HV1	P1+HV1
Intracellular viral DNA	[2]	[2]	[2]	.....	

[1] See exception given at asterisk at end of appendix D.
[2] See text.

III–A–2–a–(2)–(c)–(*2*)–(*b*). P2 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole genome, or (ii) purified cDNA copies of viral mRNA.*(37)*

III–A–2–a–(2)–(d). *Double-Stranded Segmented RNA Viruses.* P1 physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) mixtures of subgenomic cDNA segments, (ii) a specific subgenomic cDNA segment, or (iii) purified cDNA copies of viral mRNA.*(37)*

III–A–2–a–(2)–(e). *RNA Plant Viruses and Plant Viroids.* (*48*) P1l physical containment + an HV1 host-vector shall be used for DNA recombinants produced with (i) cDNA copies of the whole viral genome, (ii) subgenomic cDNA segments, or (iii) purified cDNA copies of viral mRNA.*(37)*

III–A–2–a–(3). *Intracellular Viral DNA.* Physical and biological containment specified for shotgun experiments with eukaryotic cellular DNA [see Section III–A–(1)–(a)] shall be used for DNA recombinants produced with integrated viral DNA or viral genomes present in infected cells.

III–A–2–b. *Eukaryotic Organelle DNAs.* P2 phycial containment + an HV1 host-vector, or P1l + HV2, for mitochondrial or chloroplast DNA from eukaryotes when the organelle DNA has been obtained from isolated organelles. Otherwise, the conditions given for shotgun experiments apply.

III–A–2–c. *Prokaryotic Plasmid and Phage DNAs.* The containment levels required for shotgun experiments with DNA from prokaryotes apply to their plasmids or phages (See Section III–A–1–b.)

III–A–3. *Lowering of Containment Levels for Characterized or Purified DNA Preparations and Clones.* Many of the risks which might conceivably arise from some types of recombinant DNA experiments, particularly shotgun experiments, would result from the inadvertent cloning of a harmful sequence. Therefore, in cases where the risk of inadvertently cloning the "wrong" DNA is reduced by prior enrichment for the desired piece, or in which a clone made from a random assortment of DNAs has been purified and the absence of harmful sequences established, the containment conditions for further work may be reduced. The following section outlines the mechanisms for such reductions.

III–A–3–a. *Purified DNA Other than Plasmids, Bacteriophages, and Other Viruses.* The formation of DNA recombinants from cellular DNAs that have been purified (*41*) and in which the absence of harmful sequences has been established (*3*) can be carried out under lower containment conditions than used for the corresponding shotgun experiment.(*42*). The containment may be decreased one step in physical containment (P4+P3; P3+P2; P2+P1) while maintaining the biological containment specified for the shotgun experiment, or one step in biological containment (HV3+HV2; HV2+HV1) while maintaining the specified physical containment. The Institutional Biosafety Committee (IBC) must review such a reduction and the approval of the IBC and of the NIH must be secured before such a reduction may be put into effect. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–a to levels below P1+HV1, which requires prior NIH approval. (See Section IV–E–1–b–(3)–(e).)

III–A–3–b. *Characterized Clones of DNA Recombinants.* When a cloned DNA recombinant has been rigorously characterized and the absence of harmful sequences has been established (*3*) experiments involving this recombinant DNA may be carried out under lower containment conditions. Institutional Biosafety Committees (IBCs) may give approval for a single-step reduction in physical or biological containment on receipt of evidence of characterization of a clone derived from a shotgun experiment and its probable freedom from harmful genes. IBC approval is sufficient for such a reduction except for any lowering of containment under Section III–A–3–b to levels below P1+HV1, or reduction of containment levels by more than one step, which also requires prior NIH approval. (See Section IV–E–1–b–3–(e).)

III–B. *Experiments with Prokaryotic Host-Vectors Other Than E. coli K–12*

III–B–1. *HV1 and HV2 Systems.* Certain certified HV1 and HV2 host-vector systems appear in Appendix D. The containment levels for these systems are given in the subsections of Section III–A. Other systems in the future may be certified as HV1 and HV2. At the time of certification, the classification of containment levels for experiments using them will be assigned by NIH.

III–B–2. *Return of DNA Segments to Prokaryotic Non-HV1 Host of Origin.* Certain experiments involving those prokaryotes that echange genetic information with *E. coli* by known physiological processes will be exempt from these Guidelines if they appear on the "list of exchangers" set forth in Appendix A (see Section I–E–4). For a prokaryote which can exchange genetic information(*35*) with *E. coli* under laboratory conditions but which is not on the list (Host A), the following type of experiment may be carried out under P1 conditions without Host A having been approved as an HV1 host: DNA from Host A may be inserted into a vector and propagated in *E. coli* K–12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host A by mobilization, transformation, or transduction and may then be propagated in Host A in any desired vector under P1 conditions.

For a prokaryote which does not exchange genetic information with *E. coli* (Host B), the following type of experiment may be carried out without Host B having been approved as an HV1 host: DNA from Host B may be inserted into a vector and propagated in *E. coli* K–12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host B and progagated in Host B under P1 conditions.(*43*)

III–B–3. *Non-HV1 Systems.* Containment levels for other classes of experiments involving non-HV1 systems may be approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).)

In those cases where genetic exchange has not been demonstrated between two bacterial species A and B, neither of which is known to be pathogenic for man, animals, or plants, recombinant DNA experiments involving only A and B can be conducted under P3 containment.(*2A*) Lower levels of physical containment may be assigned by NIH for specific donor-recipient combinations (See Section IV–E–1–b–2–(f)).

III–C. *Experiments with Eukaryotic Host-Vectors.*

III–C–1. *Vertebrate Host-Vector System. (44)* The subsections of Sections III–C–1–a, –b, –c and –d involve the use of specific viral vectors, namely polyoma, SV40, human adenoviruese 2 and 5, and mouse adenovirus strain FL, respectively. The subsections of Section III–C–1–e involve the use of all viral vectors including the specific viral vectors considered in the subsections of Sections III–C–1–a, –b, –c and –d, as well as any other viral vector. When the reader finds that the containment level given for specific experiment in a subsection of Section III–C–1–e is different from the containment level given in a subsection of Section III–C–1–a, –b, –c or –d, he may choose which of the two containment levels he wishes to use for the experiment.

III–C–1–a. *Polyoma Virus.*

III–C–1–a–(1). *Productive Virus-Cell Interactions.*

III–C–1–a–(1)–(a). Defective or whole polyoma virus genomes, with appropriate helper, if necessary, can be used in P2 conditions to propagate DNA sequences:

III–C–1–a–(1)–(a)–(*1*). from bacteria of Class 1 or Class 2 (*1*) or their phages or plasmids, except for those that produce potent polypeptide toxins; (*34*)

III–C–1–a–(1)–(a)–(*2*). from mice;

III–C–1–a–(1)–(a)–(*3*). from eukaryotic organisms that do not produce potent polypeptide toxins, (*34*) provided that the DNA segment is > 99% pure.

III–C–1–a–(1)–(b). Defective polyoma genomes with appropriate helper, if necessary, can be used in P2 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins.(*34)*

III–C–1–a–(1)–(C). Whole virus genomes with appropriate helper, if necessary, can be used in P3 conditions for shotgun experiments to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins.(*34).*

III–C–1–a–(1)–(d). Experiments involving the use of defective polyoma virus genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–a–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole polyoma virus genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the precribed physical and biological containment conditions (See Section IV–E–1–b–(3)–(c).)

III–C–1–b. *Simian Virus 40.*

III–C–1–b–(1). *Productive Virus-Cell Interactions.*

III–C–1–b–(1)–(a). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III–C–1–b–(1)–(a)–(*1*). bacteria of Class 1 or Class 2,(*1*) or their phages or plasmids, except for those that produce potent polypeptide toxins;(*34*)

III–C–1–b–(1)–(a)–(*2*). unifected African green monkey kidney cell cultures.

III–C–1–b–(1)–(b). SV40 DNA, rendered unconditionally defective by a deletion in an essential gene with an appropriate helper, can be used in P3 conditions to propagate DNA sequences from eukaryotic organisms that do not produce potent polypeptide toxins(*34*) (Shotgun experiments or purified DNA).

III–C–1–b–(1)–(c). Experiments involving the use of defective SV40 genomes to progagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–b–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole SV40 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNS sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis(*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–c. *Human Adenoviruses 2 and 5.*

III–C–1–c–(1). *Productive Virus-Cell Interactions.*

III–C–1–c–(1)–(a). Human adenoviruses 2 and 5, rendered unconditionally defective by deletion of at least two essential genes, with appropriate helper, can be used in P3 conditions to propagate DNA sequences from:

III–C–1–c–(1)–(a)–(*1*). Bacteria of Class 1 or Class 2 (*1*) or their phages or plasmids except for those that produce potent ploypeptide toxins; (*34*).

III–C–1–c–(1)–(a)–(2). Eukaryotic organisms that do not produce potent polypeptide toxins (*34*) (shotgun experiments or purified DNA).

III–C–1–c–(1)–(b). Experiments involving the use of unconditionally defective human adenovirus 2 and 5 genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–c–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole human adenovirus 2 and 5 genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–d. *Murine Adenovirus Strain FL.*

III–C–1–d–(1). *Productive Virus-Cell Interactions.*

III–C–1–d–(1)–(a). Unconditionally defective murine adenovirus strain FL genomes, with appropriate helper, can be used in P2 conditions to propagate DNA sequences from:

III–C–1–d–(1)–(a)–(*1*). Bacteria of Class 1 or Class 2 (*1*) or their phages or plasmids except for those that produce potent polypeptide toxins; (*34*).

III–C–1–d–(1)–(a)–(*2*). Eukaryotic organisms that do not produce potent polypeptide toxins (*34*) (shotgun experiments or purified DNA).

III–C–1–d–(1)–(b). Experiments involving the use of whole murine adenovirus strain FL genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–d–(1)–(c). Experiments involving the use of unconditionally defective murine adenovirus strain FL genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–d–(2). *Nonproductive Virus-Cell Interactions.* Defective or whole murine adenovirus strain FL genomes can be used as vectors in P2 conditions when production of viral particles cannot occur (e.g., transformation of nonpermissive cells or propagation of an unconditionally defective recombinant genome in the absence of helper), provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated by NIH on a case-by-case basis (*45*) and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–1–e. All Viral Vectors.

III–C–1–e–(1). Other experiments involving eukaryotic virus vectors can be done as follows:

III–C–1–e–(1)–(a). Recombinant DNA molecules containing no more that two-thirds of the genome of any eukaryotic virus [all viruses from a single Family (*36*) being considered identical (*50*)] may be propagated and maintained in cells in tissue culture using P1 containment. For such experiments, it must be shown that the cells lack helper virus for the specific Families of defective viruses being used. The DNA may contain fragments of the genomes of viruses from more than one Family but each fragment must be less than two-thirds of a genome.

III–C–1–e–(1)–(b). Recombinants with less than two-thirds of the genome of any eukaryotic virus may be rescued with a helper virus using P2 containment if wild type strains of the virus are CDC Class 1 or 2 agents, or using P3 containment if wild type strains of the virus are CDC Class 3 agents *(1).*

III–C–1–e–(2). Experiments involving the use of other whole or defective virus genomes to propagate DNA sequences from prokaryotic or eukaryotic organisms (and viruses), or as vectors to transform nonpermissive cells, will be evaluated by NIH on a case-by-case basis *(45)* and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

NIH will also review on a case-by-case basis *(45)* all experiments involving the use of virus vectors in animals and will prescribe the physical and biological containment conditions appropriate for such studies. (See Section IV–E–1–b–(3)–(c).)

III–C–1–f. *Nonviral Vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that hose (or a closely related strain of the same species), are exempt from these Guidelines (see Section I–E). DNA recombinants formed between such vectors and nonviral DNA from cells other than the host species require only P1 physical containment for cells in culture since vertebrate cells in tissue culture inherently exhibit a very high level of containment. Recombinants involving viral DNA or experiments which require the use of the whole animals will be evaluated by NIH on a case-by-case basis. *(45)*

III–C–2. *Invertebrate Host-Vector Systems.*

III–C–2–a. *Insect Viral Vectors.* As soon as information becomes available on the host range restrictions and on the infectivity, persistence, and integration of the viral DNA in vertebrate and invertebrate cells, experiments involving the use of insect viruses to propagate DNA sequences will be evaluated by NIH on a case-by-case basis *(45)* and will be conducted under the recommended physical containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–2–b. *Nonviral Vectors.* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these Guidelines (See Section I–E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P1 physical containment for invertebrate cells in culture since invertebrate cells in culture inherently exhibit a very high level of containment. Experiments which require the use of whole animals will be evaluated by NIH on a case-by-case basis. *(45)*

III–C–3. *Plant Viral Host-Vector Systems. (48)* The DNA plant viruses which could currently serve as vectors for cloning genes in plants and plant cell protoplasts are Cauliflower Mosiac Virus (CaMV) and its close relatives *(2A)* which have relaxed circular double-stranded DNA genomes with a molecular weight of 4.5×10^6, and Bean Golden Mosaic Virus (BGMV) and related viruses with small ($<10^6$ daltons) single-stranded DNA genomes. CaMV is spread in nature by aphids, in which it survives for a few hours. Spontaneous mutants of CaMV which lack a factor essential for aphid transmission arise frequently. BGMV is spread in nature by whiteflies, and certain other single-stranded DNA plant viruses are transmitted by leafhoppers.

The DNA plant viruses have narrow host ranges and are relatively difficult to transmit mechanically to plants. For this reason, they are most unlikely to be accidentally transmitted from spillage of purified virus preparations.

When these viruses are used as vectors in intact plants, or propagative plant parts, the plants shall be grown under P1 conditions—that is, in either a limited access greenhouse or plant growth cabinet which is insect-restrictive, preferably with positive air pressure, *(2A)* and in which an insect fumigation regime is maintained. Soil, plant pots, and unwanted infected materials shall be removed from the greenhouse or cabinet in sealed insect-proof containers and sterilized. It is not necessary to sterilize run-off water from the infected plants, as this is not a plausible route for secondary infection. When the viruses are used as vectors in tissue cultures or in small plants in axenic cultures, no special containment is necessary.

Infected plant materials which have to be removed from the greenhouse or cabinet for further research shall be maintained under insect-restrictive conditions. These measures provide an entirely adequate degree of containment. They are similar to those required in many countries for licensed handling of "exotic" plant viruses.

The viruses or their DNA may also be useful as vectors to introduce genes into plant protoplasts. The fragility of plant protoplasts combined with the properties of the viruses provides adequate safety. Since no risk to the environment from the use of the DNA plant virus/protoplast system is envisaged, no special containment is necessary, except as described in the following paragraph.

Experiments involving the use of plant genomes to propagate DNA sequences from eukaryotic viruses will be evaluated by NIH on a case-by-case basis *(45)* and will be conducted under the prescribed physical and biological containment conditions. (See Section IV–E–1–b–(3)–(c).)

III–C–4. *Plant Host-Vector Systems Other than Viruses. (48)* Organelle, plasmid, and chromosomal DNAs may be used as vectors. DNA recombinants formed between such vectors and host DNA, when propagated only in that host (or a closely related strain of the same species), are exempt from these guidelines (see Section I–E). DNA recombinants formed between such vectors and DNA from cells other than the host species require P2 physical containment. The development of host-vector systems that exhibit a high level of biological containment, such as those using protoplasts or undifferentiated cells in culture, permit *(2A)* a decrease in the physical containment to P1.

Intact plants or propagative plant parts which cannot be grown in a standard P2 laboratory because of their large size may be grown under the P1 conditions described above in Section III–C–3, except that (i) sterilization of run-off water is required where this is a plausible route for secondary infection and (ii) the standard P2 practices are adopted for microbiological work, and (iii) negative air pressure should be employed in the greenhouse or growth chamber when infectious agents are used which generate airborne propagules.

III–C–5. *Fungal or Similar Lower Eukaryotic Host-Vector Systems.*

Certain certified HV1 and HV2 host-vector system appear in Appendix D. The containment levels for these systems are given in the subsections of Section III–A. Other systems in the future may be certified as HV1 and HV2. At the time of certification, they may be added to Appendix D (and thus the containment levels for their use will be those of the subsections of Section III–A). Alternatively, at the time of their certification, another classification of containment levels for experiments using them may be assigned by NIH.

In addition to the experiments described above, the following experiments may be carried out without the eukaryotic host (Host C) having been approved as an HV1 host: DNA from Host C may be inserted into a vector and propagated in *E. coli* K–12 under P1 conditions. Subsequently, this recombinant DNA may be returned to Host C and propagated there under P1 conditions. *(43)*

Containment levels for other classes of experiments involving non-HV1 systems may be expressly approved by the Director, NIH. (See Sections IV–E–1–b–(1)–(b), IV–E–1–b–(2)–(c), and IV–E–1–b–(3)–(b).)

III–C–6. *Return of DNA Segments to a Higher Eukaryotic Host of Origin.* DNA from a higher eukaryote (Host D) may be inserted into a vector and propagated in *E. coli* K–12 under P1 containment conditions. Subsequently, this recombinant DNA may be returned to Host D and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study. *(2A)*

III–C–7. *Transfer of cloned DNA Segments to Eukaryotic Organisms.*

III–C–7–a. *Transfer to Non-human Vertebrates.* DNA from any nonprohibited source [Section I–D], except for greater than one quarter of a eukaryotic viral genome, which has been cloned and propagated in *E. coli* under P1 conditions, may be transferred with the *E. coli* vector used for cloning to any eukaryotic cells in culture or to any non-human vertebrate organism and propagated under conditions of physical containment comparable to P1 and appropriate to the organism under study *(2A)*. Transfers to any other host will be considered by the RAC on a case-by-case basis *(45)*.

III–C–7–b. *Transfer to Higher Plants.* DNA from any nonprohibited source [Section I–D] which has been cloned and propagated in *E. coli* or *S. cerevisiae* under P1 conditions, may be transferred with the *E. coli* or *S. cerevisiae* vector used for cloning to any higher plant organisms (Angiosperms and Gymnosperms) and progagated under conditions of physical containment comparable to P1 and appropriate to the organism under study (*2A*). Intact plants or propagative plant parts may be grown undr P1 conditions described undr Section III–C–3. Containment must be modified to ensure that the spread of pollen, seed or other propagules is prevented. This can be accomplished by conversion to negative pressure in the growth cabinet or greenhouse or by physical entrapment by "bagging" of reproductive structures. Transfers to any other plant organisms will be considered on a case-by-case basis (*45*).

III–D. *Complementary DNAs.* Specific containment levels are given in Section III–A–2–a (see also last column of Table III) for complementary DNA (cDNA) of viral mRNA. For the other Sections of the Guidelines, where applicable, cDNAs synthesized *in vitro* are included within each of the above classifications. For example, cDNAs formed from cellular RNAs that are not purified and characterized are included under III–A–1, shotgun experiments; cDN's formed from purified and characterized RNAs are included under III–A–3; etc.

Due to the possibility of nucleic acid contamination of enzyme preparations used in the preparation of cDNAs, the investigator must employ purified enzyme preparations that are free of viral nucleic acid.

III–E. *Synthetic DNAs.* If the synthetic DNA segment is likely to (*2A*) yield a potentially harmful polynucleotide or polypeptide (e.g., a toxin or a pharmacologically active agent), the containment conditions must be as stringent as would be used for propagating the natural DNA counterpart.

If the synthetic DNA sequence codes for a harmless product,(2A) it may be propagated at the same containment level as its purified natural DNA counterpart. For example, a synthetic DNA segment which corresponds to a nonharmful gene of birds, to be propagated in *Saccharomyces cerevisiae*, would require P2 physical containment plus an HVI host-vector, or P1+ HV2.

If the synthetic DNA segment is not expressed *in vivo* as a polynucleotide or polypeptide product, the organisms containing the recombinant DNA molecule are exempt (*4*) from the Guidelines.

IV. Roles and Responsibilities

IV–A. *Policy.* Safety in activities involving recombinant DNA depends on the individual conducting them. The Guidelines cannot anticipate every possible situation. Motivation and good judgement are the key essentials to protection of health and the environment.

The Guidelines are intended to help the Institution, the Institutional Biosafety Committee (IBC), the Biological Safety Officer, and the Principal Investigator determine the safeguards that should be implemented. These Guidelines will never be complete or final, since all conceivable experiments involving recombinant DNA cannot be foreseen. Therefore, it is the responsibility of the Institution and those associated with it to adhere to the *purpose* of the Guidelines as well as to their speifics.

Each Institution (and the IBC acting on its behalf) is responsible for ensuring that recombinant DNA activities comply with the Guidelines. General recognition of institutional authority and responsibility properly establishes accountability for safe conduct of the research at the local level.

The following roles and responsibilities constitute an administrative framework in which safety is an essential and integral part of research involving recombinant DNA molecules. Further clarifications and interpretations of roles and responsibilities will be issued by NIH as necessary.

IV–B. *General Applicability.* The Guidelines are applicable to all recombinant DNA research within the United States or its territories which is conducted at or sponsored by an Institution that receives any support for recombinant DNA research from NIH. This includes research performed by NIH directly.

An individual receiving support for research involving recombinant DNA must be associated with or sponsored by an Institution that can and does assume the responsibilities assigned in these Guidelines.

The Guidelines are also applicable to projects done abroad if they are supported by NIH funds. If the host country, however, has established rules for the conduct of recombinant DNA projects, then a certificate of compliance with those rules may be submitted to NIH in lieu of compliance with the NIH Guidelines. NIH reserves the right to withhold funding if the safety practices to be employed abroad are not reasonably consistent with the NIH Guidelines.

IV–C. *General Definitions.* The following terms, which are used throughout the Guidelines, are defined as follows:

IV–C–1. "DNA" means deoxyribonucleic acid.

IV–C–2. "Recombinant DNA" or "recombinant DNA molecules" means either (i) molecules which are constructed outside living cells by joining natural or synthetic DNA segments to DNA molecules that can replicate in a living cell, or (ii) DNA molecules which result from the replication of a molecule described in (i) above.

IV–C–3. (Deleted)

IV–C–4. "Institution" means any public or private entity (including Federal, State, and local government agencies).

IV–C–5. "Institutional Biosafety Committee" or "IBC" means a committee that (i) meets the requirements for membership specified in Section IV–D–2, and (ii) reviews, approves, and oversees projects in accordance with the responsibilities defined in Sections IV–D–2 and –3.

IV–C–6. "NIH Office of Recombinant DNA Activities" or "ORDA" means the office within NIH with responsibility for (i) reviewing and coordinating all activities of NIH related to the Guidelines, and (ii) performing other duties as defined in Section IV–E–3.

IV–C–7. "Recombinant DNA Advisory Committee" or "RAC" means the public advisory committee that advises the Secretary, the Assistant Secretary for Health, and the Director of the National Institutes of Health concerning recombinant DNA research. The RAC shall be constituted as specific in Section IV–E–2.

IV–C–8. "Director, NIH" or "Director" means the Director of the National Institutes of Health and any other officer or employee of NIH to whom authority has been delegated.

IV–C–9. "Federal Interagency Advisory Committee on Recombinant DNA Research" means the committee established in October 1976 to advise the Secretary, HEW, the Assistant Secretary for Health, and the Director, NIH, on the coordination of those aspects of all Federal programs and activities which relate to recombinant DNA research.

IV–C–10. "Administrative Practices Supplement" or "APS" means a publication to accompany the NIH Guidelines specifying administrative procedures for use at NIH and at Institutions.

IV–C–11. "Laboratory Safety Monograph" or "LSM" means a publication to accompany the NIH Guidelines describing practices, equipment, and facilities in detail.

IV–D. *Responsibilities of the Institution.*

IV–D–1. Each Institution conducting or sponsoring recombinant DNA research covered by these Guidelines is responsible for ensuring that the research is carried out in full conformity with the provisions of the Guidelines. In order to fulfill this responsibility, the Institution shall:

IV–D–1–a. Establish and implement policies that provide for the safe conduct of recombinant DNA research and that ensure compliance with the Guidelines. The Institution, as part of its general responsibilities for implementing the Guidelines, may establish additional procedures, as deemed necessary, to govern the Institution and its components in the discharge of its responsibilities under the Guidelines. This may include (i) statements formulated by the Institution for general implementation of the Guidelines and (ii) whatever additional precautionary steps the Institution may deem appropriate.

IV–D–1–b. Establish an Institutional Biosafety Committee (IBC) that meets the requirements set forth in Section IV–D–2 and carries out the functions detailed in Section IV–D–3.

IV–D–1–c. (Deleted)

IV–D–1–d. (Deleted)

IV–D–1–e. If the Institution is engaged in recombinant DNA research at the P3 or P4 containment level, appoint a Biological Safety Officer (BSO), who shall be a member of the IBC and carry out the duties specified in Section> IV–D–4.

IV–D–1–f. Require that investigators responsible for research covered by these Guidelines comply with the provisions of Section IV–D–5, and assist investigators to do so.

IV–D–1–g. Ensure appropriate training for the IBC chairperson and members, the BSO, Principal Investigators (PIs), and laboratory staff regarding the Guidelines, their implementation, and laboratory safety. Responsibility for training IBC members may be carried out through the IBC chairperson. Responsibility for training laboratory staff may be carried out through the PI. The Institution is responsible for seeing that the PI has sufficient training, but may delegate this responsibility to the IBC.

IV–D–1–h. Determine the necessity, in connection with each project, for health surveillance of recombinant DNA research personnel, and conduct, if found appropriate, a health surveillance program for the project. [The Laboratory Safety Monograph (LSM) discusses various possible components of such a program—for example, records of agents handled, active investigation of relevant illnesses, and the maintenance of serial serum samples for monitoring serologic changes that may result from the employees' work experience. Certain medical conditions may place a laboratory worker at increased risk in any endeavor where infectious agents are handled. Examples given in the LSM include gastrointestinal disorders and treatment with steroids, immunosuppressive drugs, or antibiotics. Workers with such disorders or treatment should be evaluated to determine whether they should be engaged in research with potentially hazardous organisms during their treatment or illness.]

IV–D–1–i. Report within 30days to ORDA any significant problems with and violations of the Guidelines and significant research-related accidents and illnesses, unless the institution determines that the PI or IBC has done so.

IV–D–2. *Membership and Procedures of the IBC.* The Institution shall establish an Institutional Biosafety Committee (IBC) meeting the following requirements:

IV–D–2–a. The IBC shall comprise no fewer than five members so selected that they collectively have experience and expertise in recombinant DNA technology and the capability to assess the safety of recombinant DNA research experiments and any poitential risk to public health or the environment. At least two members (but not less than 20 percent of the membership of the committee) shall not be affiliated with the Institution (apart from their membership on the IBC) and shall represent the interest of the surrounding community with respect to health and protection of the environment. Members meet this requirement if, for example, they are officials of State or local public health or environmental protection agencies, members of other local governmental bodies, or persons active in medical, occupational health, or environmental concerns in the community The Biological Safety Officer (BSO), mandatory when research is being conducted at the P3 and P4 levels, shall be a member (see Section IV–D–4).

IV–D–2–b. In order to ensure the professional competence necessary to review recombinant DNA activities, it is recommended that (i) the IBC include persons from disciplines relevant to recombinant DNA technology, biological safety, and engineering; (ii) the IBC include, or have available as consultants, persons knowledgeable in institution commitments and policies, applicable law, standards of professional conduct and practice, community attitudes, and the environment; and (iii) at least one member be a nondoctoral person from a laboratory technical staff.

IV–D–2–c. The Institution shall identify the committee members by name in a report to the NIH Office of Recombinant DNA Activities (ORDA) and shall include relevant background information on each member in such form and at such times as ORDA may require. (See the Administrative Practice Supplement for further guidance.)

IV–D–2–d. No member of an IBC may be involved (except to provide information requested by the IBC) in the review or approval of a project in which he or she has been, or expects to be, engaged or has a direct financial interest.

IV–D–2–e. The Institution may establish procedures that the IBC will follow in its initial and continuing review of applications, proposals, and activities. (IBC review procedures are specified in Section IV–D–3–a.)

IV–D–2–f. Central to implementation of the Guidelines is the review of experiments by the IBC. In carrying out this responsibility, the Institution shall comply with instructions and procedures specified in the Administrative Practices Supplement.

IV–D–2–g. Institutions are encouraged to open IBC meetings to the public whenever possible, consistent with protection of privacy and proprietary interests.

IV–D–2–h. Upon request, the Institution shall make available to the public all minutes of IBC meetings and any documents submitted to or received from funding agencies which the latter are required to make available to the public (e.g., reports of Guideline violations and significant research-related accidents, and agency directives to modify projects). If comments are made by members of the public on IBC actions, the Institution shall forward to NIH both the comments and the IBC's response.

IV–D–3. *Functions of the IBC.* On behalf of the Institution, the IBC is responsible for:

IV–D–3–a. Reviewing for compliance with the NIH Guidelines all recombinant DNA research conducted at or sponsored by the Institution, and approving those research projects that it finds are in conformity with the Guidelines. This review shall include:

IV–D–3–a–(1). An independent assessment of the containment levels required by these Guidelines for the proposed research, and

IV–D–3–a–(2). An assessment of the facilities, procedures, and practices, and of the training and expertise of recombinant DNA personnel.

Note.—See Laboratory Safety Monograph (pages 187–190) for suggested guidance in conducting this review.

IV–D–3–b. Notifying the Principal Investigator (PI) of the results of their review.

IV–D–3–c. Reviewing periodically recombinant DNA research being conducted at the Institution, to ensure that the requirements of the Guidelines are being fulfilled.

IV–D–3–d. Adopting emergency plans covering accidental spills and personnel contamination resulting from such research.

Note.—Basic elements in developing specific procedures for dealing with major spills of potentially hazardous materials in the laboratory are detailed in the Laboratory Safety Monograph. Included are information and references on decontamination and emergency plans. NIH and the Centers for Disease Control are available to provide consultation, and direct assistance if necessary, as posted in the LSM. The Institution shall cooperate with the State and local public health departments, reporting any significant reseach-related illness or accident that appears to be a hazard to the public health.

IV–D–3–e. Reporting within 30 days to the appropriate institutional official and to the NIH Office of Recombinant DNA Activities (ORDA) any significant problems with or violations of the Gidelines, and any significant research-related accidents or illnesses, unless the IBC determines that the PI has done so.

IV–D–3–f. The IBC may not authorize initiation of experiments not explicitly covered by the Guidelines until NIH, (with the advice of the RAC when required) establishes the containment requirement.

IV–D–3–g. Performing such other functions as may be delegated to the IBC under Section IV–D–1.

IV–D–4. *Biological Safety Officer.* The Institution shall appoint a BSO if it engages in recombinant DNA research at the P3 or P4 containment level. The officer shall be a member of the Institutional Biosafety Committee (IBC), and his or her duties shall include (but need not be limited to):

IV–D–4–a. Ensuring through periodic inspections that laboratory standards are rigorously followed;

IV–D–4–b. Reporting to the IBC and the Institution all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses of which the BSO becomes aware, unless the BSO determines that the Principal Investigator (PI) has done so;

IV–D–4–c. Developing emergency plans for dealing with accidental spills and personnel contamination, and investigating recombinant DNA research laboratory accidents;

IV–D–4–d. Providing advice on laboratory security;

IV–D–4–e. Providing technical advice to the PI and the IBC on research safety procedures.

Note.—See Laboratory Safety Monograph for additional information on the duties of the BSO.

IV–D–5. *Principal Investigator.* On behalf of the Institution, the PI is responsible for complying fully with the Guidelines in conducting any recombinant DNA research.

IV–D–5–a. *PI—General.* As part of this general responsibility, the PI shall:

IV–D–5–a–(1). Initiate or modify no recombinant DNA research subject to the Guidelines until that research, or the proposed modification thereof, has been approved by the Institutional Biosafety Committee (IBC) and has met all other requirements of the Guidelines and the Administrative Practices Supplement (APS).

(Note.—No prior approval by the IBC is required for most experiments described in Section III–O. Modify containment and experimental protocol according to recommendations of the IBC.)

IV–D–5–a–(2). Report within 30 days to the IBC and NIH (ORDA) all significant problems with and violations of the Guidelines and all significant research-related accidents and illnesses;

IV–D–5–a–(3). Report to the IBC and to NIH (ORDA) new information bearing on the Guidelines;

IV–D–5–a–(4). Be adequately trained in good microbiological techniques;

IV–D–5–a–(5). Adhere to IBC-approved emergency plans for dealing with accidental spills and personnel contamination; and

IV–D–5–a–(6). Comply with shipping requirements for recombinant DNA molecules. (See Section II–C for shipping requirements, Laboratory Safety Monograph for technical recommendations, and the APS for administrative instructions and procedures. The requesting laboratory must be in compliance with the NIH Guidelines and under appropriate review by its IBC, and the sending investigator must maintain a record of all shipments of recombinant DNA materials.)

IV–D–5–b. *Submissions by the PI to NIH.* The PI shall:

IV–D–5–b–(1). Submit information to NIH (ORDA) in order to have new host-vector systems certified;

IV–D–5–b–(2). Petition NIH, with notice to the IBC, for exemptions to these Guidelines (see Sections I–E–4 and I–E–5 and, for additional information on procedures, the APS); and

IV–D–5–b–(3). Petition NIH, with concurrence of the IBC, for exceptions to the prohibitions under these Guidelines (see Section I–D and, for additional information on procedures, the APS).

IV–D–5–b–(4). Petition NIH for determination of containment for experiments requiring case-by-case review.

IV–D–5–b–(5). Petition NIH for determination of containment for experiments not covered by the Guidelines.

IV–D–5–c. *Submissions by the PI to the IBC.* The PI shall:

IV–D–5–c–(1). Make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines;

IV–D–5–c–(2). Select appropriate microbiological practices and laboratory techniques to be used in the research;

IV–D–5–c–(3). Submit the initial research protocol (and also subsequent changes—e.g., changes in the source of DNA or host-vector system) to the IBC for review and approval or disapproval, and

IV–D–5–c–(4). Remain in communication with the IBC throughout the conduct of the project.

IV–D–5–d. *PI Responsibilities After Approval but Prior to Initiating the Research.* The PI is responsible for:

IV–D–5–d–(1). Making available to the laboratory staff copies of the approved protocols that describe the potential biohazards and the precautions to be taken;

IV–D–5–d–(2). Instructing and training staff in the practices and techniques required to ensure safety and in the procedures for dealing with accidents; and

IV–D–5–d–(3). Informing the staff of the reasons and provisions for any precautionary medical practices advised or requested, such as vaccinations or serum collection.

IV–D–5–e. *PI Responsibilities During the Conduct of the Approved Research.* The PI is responsible for:

IV–D–5–e–(1). Supervising the safety performance of the staff to ensure that the required safety practices and techniques are employed;

IV–D–5–e–(2). Investigating and reporting in writing to ORDA, the Biological Safety Officer (where applicable), and the IBC any significant problems pertaining to the operation and implementation of containment practices and procedures;

IV–D–5–e–(3). Correcting work errors and conditions that may result in the release of recombinant DNA materials;

IV–D–5–e–(4). Ensuring the integrity of the physical containment (e.g., biological safety cabinets) and the biological containment (e.g., purity, and genotypic and phenotypic characteristics); and

IV–D–5–e–(5). *Publications.* PIs are urged to include, in all publications reporting on recombinant DNA research, a description of the physical and biological containment procedures employed.

IV–E. *Responsibilities of NIH.*

IV–E–1. *Director.* The Director, NIH, is responsible for (i) establishing the NIH Guidelines on recombinant DNA research, (ii) overseeing their implementation, and (iii) their final interpretation.

The Director has a number of responsibilities under the Guidelines that involve the NIH Office of Recombinant DNA Activities (ORDA) and the Recombinant DNA Advisory Committee (RAC). ORDA's responsibilities under the Guidelines are administrative. Advice from the RAC is primarily scientific and technical. In certain circumstances, there is specific opportunity for public comment, with published response, before final action.

IV–E–1–a. *General Responsibilities of the Director, NIH.* The responsibilities of the Director shall include the following:

IV–E–1–a–(1). Promulgating requirements as necessary to implement the Guidelines;

IV–E–1–a–(2). Establishing and maintaining the RAC to carry out the responsibilities set forth in Section IV–E–2. The RAC's membership is specified in its charter and in Section IV–E–2;

IV–E–1–a–(3). Establishing and maintaining ORDA to carry out the responsibilities defined in Section IV–E–3; and

IV–E–1–a–(4). Maintaining the Federal Interagency Advisory Committee on Recombinant DNA Research established by the Secretary, HEW, for advice on the coordination of all Federal programs and activities relating to recombinant DNA, including activities of the RAC.

IV–E–1–b. *Specific Responsibilities of the Director, NIH.* In carrying out the responsibilities set forth in this Section, the Director shall weigh each proposed action, through appropriate analysis and consultation, to determine that it complies with the Guidelines and presents no significant risk to health or the environment.

IV–E–1–b–(1). *The Director is responsible for the following major actions* (For these, the Director must seek the advice of the RAC and provide an opportunity for public and Federal agency comment. Specifically, the agenda of the RAC meeting citing the major actions will be published in the **Federal Register** at least 30 days before the meeting, and the Director will also publish the proposed actions in the **Federal Register** for comment at least 30 days before the meeting. In addition, the Director's proposed decision, at his discretion, may be published in the **Federal Register** for 30 days of comment before final action is taken. The Director's final decision, along with response to the comments, will be published in the **Federal Register** and the *Recombinant DNA Technical Bulletin.* The RAC and IBC chairpersons will be notified of this decision):

IV–E–1–b–(1)–(a). Changing containment levels for types of experiments that are specified in the Guidelines when a major action is involved;

IV–E–1–b–(1)–(b). Assigning containment levels for types of experiments that are not explicitly considered in the Guidelines when a major action is involved;

IV–E–1–b–(1)–(c). Certifying new host-vector systems, with the exception of

minor modifications of already certified systems. [The standards and procedures for certification are described in Section II–D–2–a. Minor modifications constitute, for example, those of minimal or no consequence to the properties relevant to containment. See the Administrative Practices Supplement (APS) for further information];

IV–E–1–b–(1)–(d). Promulgating and amending a list of classes of recombinant DNA molecules to be exempt from these because they consist entirely of DNA segments from species that exchange DNA by known physiological processes, or otherwise do not present a significant risk to health or the environment (see Sections I–E–4 and –5 and the APS for further information);

IV–E–1–b–(1)–(e). Permitting exceptions to the prohibited experiments in the Guidelines, in order, for example, to allow risk-assessment studies; and

IV–E–1–b–(1)–(f). Adopting other changes in the Guidelines.

IV–E–1–b–(2). *The Director is also responsible for the following lesser* actions (For these, the Director must seek the advice of the RAC. The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the Recombinant DNA Technical Bulletin):

IV–E–1–b–(2)–(a). Interpreting and determining containment levels, upon request by ORDA;

IV–E–1–b–(2)–(b). Changing containment levels for experiments that are specified in the Guidelines (see Section III);

IV–E–1–b–(2)–(c). Assigning containment levels for experiments not explicitly considered in the Guidelines (see Section III);

IV–E–1–b–(2)–(d). Designating certain class 2 agents as class 1 for the purpose of these Guidelines (see Footnote 1 and Appendix B);

IV–E–1–b–(2)–(e). Assigning containment levels for experiments with recombinant DNA from Class 3 organisms(*1*) and assigning containment levels for experiments which increase the host-range and virulence of plant pathogens beyond that which occurs by natural genetic exchange; and

IV–E–1–b–(2)–(f). Assigning containment levels for experiments in which both donor and recipient are non-pathogenic prokaryotes (see Section III–B–3).

IV–E–1–b–(3). *The Director is also responsible for the following actions.* (The Director's decision will be transmitted to the RAC and IBC chairpersons and published in the *Recombinant DNA Technical Bulletin*):

IV–E–1–b–(3)–(a). Interpreting the Guidelines for experiments to which the Guidelines specifically assign containment levels;

IV–E–1–b–(3)–(b). Determining appropriate containment conditions for experiments according to case precedents developed under Section IV–E–1–b–(2)–(c).

IV–E–1–b–(3)–(c). Determining appropriate containment conditions upon case-by-case analysis of experiments explicity considered in the Guidelines but for which no containment levels have been set (see Footnote 45 in Part V; Sections III–C–1–a through –e; and Sections III–C–2 and –3);

IV–E–1–b–(3)–(d). Authorizing, under procedures specified by the RAC, large-scale experiments (i.e., involving more than 10 liters of culture) for recombinat DNAs that are rigorously characterized and free of harmful sequences (see Footnote 3 and Section I–D–6);

IV–E–1–b–(3)–(e). Lowering containment levels for characterized clones or purified DNA (see Sections III–A–3–a and –b, and Footnotes 3 and 41);

IV–E–1–b–(3)–(f). Approving minor modifications of already certified host-vector systems. (The standards and procedures for such modifications are described in Section II–D–2); and

IV–E–1–b–(3)–(g). Decertifying already certified host-vector systems.

IV–E–1–b–(4). The Director shall conduct, support, and assist training programs in laboratory safety for Institutional Biosafety Committee members, Biological Safety Officers, Principal Investigators, and laboratory staff.

IV–E–1–b–(5). The Director, at the end of 36 months from the time these Guidelines are promulgated, will report on the Guidelines, their administration, and the potential risks and benefits of this research. In doing so, the Director will consult with the RAC and the Federal Interagency Committee. Public comment will be solicited on the draft report and taken into account in transmitting the final report to the Assistant Secretary for Health and the Secretary, HEW.

IV–E–2. *Recombinant Advisory Committee.* The NIH Recombinant DNA Advisory Committee (RAC) is responsible for carrying out specified functions cited below as well as others assigned under its charter or by the Secretary, HEW, the Assistant Secretary for Health, and the Director, NIH.

The members of the committee shall be chosen to provide, collectively, expertise in scientific fields relevant to recombinant DNA technology and biological safety—e.g., microbiology, molecular biology, virology, genetics, epidemiology, infectious diseases, the biology of enteric organisms, botany, plant pathology, ecology, and tissue culture. At least 20 percent of the members shall be persons knowledgeable in applicable law, standards of professional conduct and practice, public attitudes, the environment, public health, occupational health, or related fields. Representatives from Federal agencies shall serve as nonvoting members. Nominations for the RAC may be submitted to the NIH Office of Recombinant DNA Activities, Bethesda, Md. 20205.

All meetings of the RAC will be announced in the **Federal Register**, including tentative agenda items, 30 days in advance of the meeting, with final agendas (if modified) available at least 72 hours before the meeting. No item defined as a major action under Section IV–E–1–b–(1) may be added to an agenda after it appears in the **Federal Register.**

IV–E–2–a. *The RAC shall be responsible for advising the Director, NIH, on the actions listed in Section IV–E–1–b–(1)* and *–(2).*

IV–E–3. *The Office of Recombinant DNA Activities.* ORDA shall serve as a focal point for information on recombinant DNA activities and provide advice to all within and outside NIH, including Institutions, Biological Safety Committee, Principal Investigators, Federal agencies, State and local governments, and institutions in the private sector. ORDA shall carry out such other functions as may be delegated to it by the Director, NIH, including those authorities described in Section IV–E–1–b–(3). In addition, ORDA shall be responsible for the following:

IV–E–3–a. Review and approval of Institutional Biosafety Committee (IBC) membership;

IV–E–3–b through IV–E–3–c–(3). (Deleted)

IV–E–3–c–(4). Publish in the **Federal Register:**

IV–E–3–c–(4)–(a). Announcements of Recombinant DNA Advisory Committee (RAC) meetings and agenda 30 days in advance, with publication of the Director's proposed decision for 30 days of public and Federal agency comment followed by a published response, on any action listed in Section IV–E–1–(b)–(1); and

IV–E–3–c–(4)–(b). Announcements of RAC meetings and agendas 30 days in advance on any action listed in Section IV–E–1–b–(2).

Note.—If the agenda for an RAC meeting is modified, ORDA shall make the revised agenda available to anyone, upon request, at least 72 hours in advance of the meeting.

IV–E–3–c–(5). Publish the *Recombinant DNA Technical Bulletin;* and

IV–E–3–c–(6). Serve as executive secretary to the RAC.

IV–E–4. *Other NIH Components.* Other NIH components shall be responsible for:

IV–E–4–a. (Deleted)

IV–E–4–b. Certifying P4 facilities, inspecting them periodically, and inspecting other recombinant DNA facilities as deemed necessary; and

IV–E–4–c. Announcing and distributing certified HV2 and HV3 host-vector systems (see Section II–E–3).

(See Administrative Practices Suplement for additional information on the administrative procedures of ORDA and other NIH components.)

IV–F. (Deleted)

IV–G. *Compliance.* As a condition for NIH funding of recombinant DNA research, institutions must ensure that such research conducted at or sponsored by the Institution, irrespective of the source of funding, shall comply with these Guidelines. The policies on noncompliance are as follows:

IV–G–1. All NIH-funded projects involving recombinant DNA techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of financial assistance for such projects and of NIH funds for other recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV–G–2. All non-NIH funded projects involving recombinant DNA techniques conducted at or sponsored by an Institution that receives NIH funds for projects involving such techniques must comply with the NIH Guidelines. Noncompliance may result in (i) suspension, limitation, or termination of NIH funds for recombinant DNA research at the Institution, or (ii) a requirement for prior NIH approval of any or all recombinant DNA projects at the Institution.

IV–G–3. Information concerning noncompliance with the Guidelines may be brought foward by any person. It should be delivered to both NIH (ORDA) and the relevant Institution. The Institution, generally through the IBC, shall take appropriate action. The Institution shall forward a complete report of the incident to ORDA, recommending any further action indicated.

IV–G–4. In cases where NIH proposes to suspend, limit, or terminate financial assistance because of noncompliance with the Guidelines, applicable DHEW and Public Health Service procedures shall govern.

IV–G–5. *Voluntary Compliance.* Any individual, corporation, or institution that is not otherwise covered by the Guidelines is encouraged to conduct recombinant DNA research activities in accordance with the Guidelines, through the procedures set forth in Part VI.

V. Footnotes And References

1. The reference to organisms as Class 1, 2, 3, 4, or 5 refers to the classification in the publication *Classification of Etiologic Agents on the Basis of Hazard,* 4th Edition, July 1974; U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333. The list of organisms in each class, as given in this publication, is reprinted in Appendix B to these Guidelines.

The Director, NIH, with advice of the Recombinant DNA Advisory Committee, may designate certain of the agents which are listed as Class 2 in the *Classification of Etiologic Agents on the Basis of Hazard,* 4th Edition, July 1974, as Class 1 agents for the Purposes of these Guidelines (See section IV–E–1–b–(2)–(d)). An updated list of such agents may be obtained from the Office of Recombinant DNA Activities (ORDA), National Institutes of Health, Bethesda, Maryland 20205.

The entire *Classification of Etiologic Agents on the Basis of Hazard* is in the process of revision.

For experiments using Vesicular Stomatitis virus (VSV), contact the NIH Office of Recombinant DNA Activities.

2A. In Parts I and III of the Guidelines, there are a number of places where Judgments are to be made. These include: "cells known to be infected with such agents" (Section I–D–1) "toxins potent for vertebrates" (Section I–D–2); "known to acquire it naturally" (Section I–D–5); "known to produce a potent polypeptide toxin * * * or known to carry such pathogens * * * not likely to be a product of closely linked eukaryote genes * * * shown not to contain such agents" (Section III–A–1–a–(5)–(a)); "shown to be free of disease causing microorganisms" (Section III–A–1–a–(5)–(b)); "close relatives" (Section III–C–3); and "procduce a potent polypeptide toxin" (Footnote 34).

In all these cases the principal investigator is to make the initial judgment on these matters as part of his responsibility to "make the initial determination of the required levels of physical and biological containment in accordance with the Guidelines" (Section IV–D–7–a). In all these cases, this judgment is to be reviewed and approved by the Institutional Biosafety Committee as part of its responsibility to make "an independent assessment of the containment levels required by these Guidelines for the proposed research" (Section IV–D–3–a–(1)). If the IBC wishes, any specific cases may be referred to the NIH Office of Recombinant DNA Activities as part of ORDA's functions to "provide advice to all within and outside NIH" (Section IV–E–3), and ORDA may request advice from the Recombinant DNA Advisory Committee as part of the RAC's responsibility for "interpreting and determining containment levels upon request by ORDA" (Section IV–E–1–b–(2)–(a)).

3. The following types of data should be considered in determining whether DNA recombinants are "characterize" and the absence of harmful sequences has been established: (a) the absence of potentially harmful genes (e.g., sequences contained in indigenous tumor viruses or sequences that code for toxins, invasins, virulence factors, etc., that might potentiate the pathogenicity or communicability of the vector and/or the host or be detrimental to humans, animals, or plants); (b) the type(s) of genetic information on the cloned sedment and the nature of transcriptional and translation gene products specified; (c) the relationship between the recovered and desired segment (e.g., hybridization and restriction endonuckease fragmentation analysis where applicable); (d) the genetic stabillity of the cloned fragment; and (e) any alterations in the biological properties of the vector and host.

4. In Section I–E, "exemptions" from the Guidelines are discussed. Such experiments are not covered by the Guidelines and need not be registered with NIH. In Section I–D on "prohibitions," the possibility of "exceptions" is discussed. An "exception" means that any experiment may be expressly released from a prohibition. At that time it will be assigned an appropriate level of physical and biological containment.

5. Care should be taken to inactivate recombinant DNA before disposal. Procedures for inactivating DNA can be found in the "Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research."

6. *Laboratory Safety at the Center for Disease Control* (Sept. 1974). U.S. Department of Health, Education, and Welfare Publication No. CDC 75–8118.

1. *Classification of Etiologic Agents on the Basis of Hazard.* (4th Edition, July 1974). U.S. Department of Health, Education and Welfare. Public Health Service. Centers for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.

8. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (Oct. 1974). U.S. Department of Health, Education and Welfare Publication No. (NIH) 75–790.

9. *National Institutes of Health Biohazards Safety Guide* (1974). U.S. Department of Health, Education, and Welfare, Public Health.

10. *Biohazards in Biological Research* (1973). A. Hellman, M. N Oxman, and R. Pollack (ed.) Cold Spring Harbor Laboratory.

11. *Handbook of Laboratory Safety* (1971). Second Edition. N. V. Steere (ed.). The Chemical Rubber Co., Cleveland.

12. Bodily, J. L. (1970). *General Administration of the Laboratory,* H. L. Bodily, E. L. Updyke, and J. O. Mason (eds.), Diagnostic Procedures for Bacterial, Mycotic and Parasitic Infections. American Public Health Association, New York, pp. 11–28.

13. Darlow, H. M. (1969). *Safety in the Microbiological Laboratory.* In J. R. Norris and D. W. Robbins (ed.), Methods in Microbiology. Academic Press, Inc. New York. pp. 169–204.

14. *The Prevention of Laboratory Acquired Infection* (1974). C. H. Collins, E. G. Hartley, and R. Pilsworth. Public Health Laboratory Service, Monograph Series No. 6.

15. Chatigny, M. A. (1961). *Protection Against Infection in the Microbiological Laboratory: Devices and Procedures.* In W. W. Umbreit (ed.). Advances in Applied Microbiology. Academic Press, New York, N.Y. 3:131–192.

16. *Design Criteria for Viral Oncology Research Facilities* (1975). U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, DHEW Publication No. (NIH) 75–891.

17. Kuehne, R. W. (1973). *Biological Containment Facility for Studying Infectious Disease.* Appl. Microbiol. 26–239–243.

18. Runkle, R. S., and G. B. Phillips (1969). *Microbial Containment Control Facilities.* Van Nostrand Reinhold, New York.

19. Chatigny, M. A., and D. I. Clinger (1969). *Contamination Control in Aerobiology.* In R. L. Dimmick and A. B. Akers (eds.). An Introduction to Experimental Aerobiology. John Wiley & Sons, New York, pp. 194–263.

19A. Horsfall, F. L., Jr., and J. H. Baner (1940). *Individual Isolation of Infected Animals in a Single Room* J. Bact. *40,* 569–580.

20. Biological safety cabinets referred to in this section are classified as *Class I, Class II,* or *Class III* cabinets. A *Class I* is a ventilated cabinet for personnel protection having an inward flow of air away from the operator. The exhaust air from this cabinet is filtered through a high-efficiency particulate air (HEPA) filter. This cabinet is used in three operational modes: (1) with a full-width open front, (2) with an installed front closure panel (having four 8-inch diameter openings) without gloves, and (3) with an installed front closure panel equipped with arm-length rubber gloves. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. A *Class II* cabinet is a ventilated cabinet for personnel and product protection having an open front with inward air flow for personnel protection, and HEPA filtered mass recirculated air flow for product protection. The cabinet exhaust air is filtered through a HEPA filter. The face velocity of the inward flow of air through the full-width open front is 75 feet per minute or greater. Design and performance specifications for *Class II* cabinets have been adopted by the National Sanitation Foundation, Ann Arbor, Michigan. A *Class III* cabinet is a closed-front ventilated cabinet of gas-tight construction which provides the highest level of personnel protection of all biohazard safety cabinets. The interior of the cabinet is protected from contaminants exterior to the cabinet. The cabinet is fitted with arm-length rubber gloves and is operated under a negative pressure of at least 0.5 inches water gauge. All supply air is filtered through HEPA filters. Exhaust air is filtered through two HEPA filters or one HEPA filter and incinerator before being discharged to the outside environment.

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29. Murray, N. E., and K. Murray (1974). *Manipulation of Restriction Targets in Phage Lambda to Form Receptor Chromosomes for DNA Fragments.* Nature *251,* 476–481.

30. Rambach, A., and P. Tiollais (1974). *Bacteriophage Having EcoRI Endonucleuse Sites Only in the Non-Essential Region of the Genome.* Proc. Nat. Acad. Sci., USA *71,* 3926–3930.

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32. Donoghue, D. J., and P. A. Sharp (1977). *An Improved Lambda Vector: Construction of Model Recombinants Coding for Kanamycin Resistance,* Gene *1,* 209–227.

33. Leder, P., D. Tiemeier and L. Enquist (1977). *EK2 Derivatives of Bacteriophage Lambda Useful in the Cloning of DNA from Higher Organisms: The gt WES System.* Science *196.* 175–177.

33A. Skalka, A. (1978). *Current Status of Coliphage EK2 Vectors.* Gene *3,* 29–35.

33B. Szybalski, W., A. Skalka, S. Gottesman, A. Campbell, and D. Botstein (1978). *Standardized Laboratory Tests for EK2 Certification.* Gene *3,* 36–38.

34. We are specifically concerned with the remote possibility that potent toxins could be produced by acquiring a single gene or cluster of genes. See also footnote 2A.

35. Defined as observable under optimal laboratory conditions by transformation, transduction, phage infection, and/or conjugation with transfer of phage, plasmid, and/or chromosomal genetic information. Note that this definition of exchange may be less stringent than that applied to exempt organisms under Section I–E–4.

36. As classified in the Third Report of the International Committee on Taxonomy of Viruses: Classification and Nomenclature of Viruses, R. E. F. Matthews, Ed. Intervirology 12 (129–296) 1979. (As noted in the Prohibition Section, the use of viruses classified [1] as Class 4 or 5 is prohibited.)

37. The cDNA copy of the viral mRNA must be >99% pure; otherwise as for shotgun experiments with eukaryotic cellular DNA.

37A. For the purpose of these Guidelines, viruses of the families *Papovavirida, Adenoviridae,* and *Herpetoviridae* (36) should be considered as "transforming" viruses. While only certain of these viruses have been associated with cell transformation *in vivo* or *in vitro,* it seems prudent to consider all members to be potentially capable of transformation. In addition, those viruses of the family *Poxviridae* that produce proliferative responses—i.e., myxoma, rabbit and squirrel fibroma, and Yaba viruses-should be considered as "transforming."

38. ≥99% pure (i.e., less than 1% of the DNA consists of intact viral genomes); otherwise as for whole genomes.

39. The viruses have been classified by NCI as "moderate-risk oncogenic viruses." See "Laboratory Safety Monograph—A Supplement to the NIH Guidelines for Recombinant DNA Research" for recommendations on handling the viruses themselves.

40. (Deleted)

41. The DNA preparation is defined as "purified" if the desired DNA represents at least 99% (w/w) of the total DNA in the preparation, provided that it was verified by more than one procedure.

42. The lowering of the containment level when this degree of purification has been obtained is based on the fact that the total number of clones that must be examined to obtain the desired clone is markedly reduced. Thus, the probability of cloning a harmful gene could, for example, be reduced by more than 10^5-fold when a nonrepetitive gene from mammals was being sought. Furthermore, the level of purity specified here makes it easier to establish that the desired DNA does not contain harmful genes.

43. This is not permitted, of course, if it falls under any of the Prohibitions of Section I–D. Of particular concern here is prohibition I–D–5, i.e., "Deliberate transfer of a drug resistance trait to micro-organisms that are not known to acquire it naturally if such acquisition could compromise the use of a drug to control disease agents in human or veterinary medicine or agriculture."

44. Because this work will be done almost exclusively in tissue culture cells, which have no capacity for propagation outside the laboratory, the primary focus for containment is the vector. It should be pointed out that risk of laboratory-acquired infection as a consequence of tissue culture manipulation is very low. Given good microbiological practices, the most likely mode of escape of recombinant DNAs from a physically contained laboratory is carriage by an infected human. Thus the vector with an inserted DNA segment should have little or no ability to replicate or spread in humans.

For use as a vector in a vertebrate host cell system, an animal viral DNA molecule should display the following properties:

(i) It should not consist of the whole genome of any agent that is infectious for humans or that replicates to a significant extent in human cells in tissue culture. If the recombinant molecule is used to transform nonpermissive cells (i.e., cells which do not produce infectious virus particles), this is not a requirement.

(ii) It should be derived from a virus whose epidemiological behavior and host range are well understood.

(iii) In permissive cells, it should be defective when carrying an inserted DNA segment (i.e., propagation of the recombinant DNA as a virus must be dependent upon the presence of a complementing helper genome). In almost all cases this condition would be achieved automatically by the manipulations used to construct and propagate the recombinants. In addition, the amount of DNA encapsidated in the particles of most animal viruses is defined within fairly close limits. The insertion of sizable foreign DNA sequences, therefore, generally demands a compensatory deletion of viral sequences. It may be possible to introduce very short insertions (50–100 base pairs) without rendering the viral vector defective. In such a situation, the requirement that the viral vector be defective is not necessary, except in those cases in which the inserted DNA encodes a biologically active polypeptide.

It is desired but not required that the functional anatomy of the vector be known—that is, fL-re sLogld be a clear idea of the location within the molecule of:

(i) the sites at which DNA synthesis originates and terminates,

(ii) the sites that are cleaved by restriction endonucleases, and

(iii) the template regions for the major gene product.

If possible the helper virus genome should:

(i) be integrated into the genome of a stable line of host cells (a situation that would effectively limit the growth of the vector recombinant to such cell lines) or

(ii) consist of a defective genome, or an appropriate conditional lethal mutant virus, making vector and helper dependent upon each other for propagation.

However, neither of these stipulations is a requirement.

45. Review of NIH on a case-by-case basis means that NIH must review and set appropriate containment conditions before the work may be undertaken. NIH actions in such case-by-case reviews will be published in the *Recombinant DNA Technical Bulletin*.

46. Provided the inserted DNA sequences are not derived from eukaryotic viruses. In the latter case, such experiments will be evaluated on a case-by-case basis.

47. >99% pure; otherwise as for shotgun experiments.

48. A USDA permit, required for import and interstate transport of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD 20782.

49. A subset of non-conjugative plasmid vectors are also poorly mobilizable (e.g., pBR322, pBR313). Where practical, these vectors should be employed.

50. i.e., the total of all genomes within a Family shall not exceed two-thirds of the genome.

VI. Voluntary Compliance

VI–A. *Basic Policy.* Individuals, corporations, and institutions not otherwise covered by the Guidelines are encouraged to do so by following the standards and procedures set forth in Parts I–IV of the Guidelines. In order to simplify discussion, references hereafter to "institutions" are intended to encompass corporations, and individuals who have no organizational affiliation. For purposes of complying with the Guidelines, an individual intending to carry out research involving recombinant DNA is encouraged to affiliate with an institution that has an Institutional Biosafety Committee approved under the Guidelines.

Since commercial organizations have special concerns, such as protection of proprietary data, some modifications and explanations of the procedures in Parts I–IV are provided below, in order to address these concerns.

VI–B. *IBC Approval.* The NIH Office of Recombinant DNA Activities (ORDA) will review the membership of an institution's Institutional Biosafety Committee (IBC) and, where it finds the IBC meets the requirements set forth in Section IV–D–2, will give its approval to the IBC membership.

It should be emphasized that employment of an IBC member solely for purposes of membership on the IBC does not itself make the member an institutionally affiliated member for purposes of Section IV–D–2–a.

Except for the unaffiliated members, a member of an IBC for an institution not otherwise covered by the Guidelines may participate in the review and approval of a project in which the member has a direct financial interest, so long as the member has not been and does not expect to be engaged in the project. Section IV–D–2–d is modified to that extent for purposes of these institutions.

VI–C. (Deleted)

VI–D. *Certification of Host-Vector Systems.* A host-vector system may be proposed for certification by the Director, NIH, in accordance with the procedures set forth in Section II–D–2–a.

Institutions not otherwise covered by the Guidelines will not be subject to Section II–D–3 by complying with these procedures.

In order to ensure protection for proprietary data, any public notice regarding a host-vector system which is designated by the institution as proprietary under Section VI–F–1 will be issued only after consultation with the institution as to the content of the notice.

VI–E. *Requests for Exceptions, Exemptions, Approvals.* Requests for exceptions from prohibitions, exemptions, or other approvals required by the Guidelines should be requested by following the procedures set forth in the appropriate sections in Parts I–IV of the Guidelines.

In order to ensure protection for proprietary data, any public notice regarding a request for an exception, exemption, or other approval which is designated by the institution as proprietary under Section VI–F–1 will be issued only after consultation with the institution as to the content of the notice.

VI–F. *Protection of Proprietary Data.* In general, the Freedom of Information Act requires Federal agencies to make their records available to the public upon request. However, this requirement does not apply to, among other things, "trade secrets and commercial and financial information obtained from a person and privileged or confidential." 18 U.S.C. 1905, in turn makes it a crime for an officer or employee of the United States or any Federal department or agency to publish, divulge, disclose, or make known "in any manner or to any extent not authorized by law any information coming to him in the course of his employment or official duties or by reason of any examination or investigation made by, or return, report or record made to or filed with, such department or agency or officer or employee thereof, which information concerns or relates to the trade secrets, [or processes * * * of any person, firm, partnership, corporation, or association." This provision applies to all employees of the Federal Government, including special Government employees. Members of the Recombinant DNA Advisory Committee are "special Government employees."

VI–F–1. In submitting information to NIH for purposes of complying voluntarily with the Guidelines, an institution may designate those items of information which the institution believes constitute trade secrets or privileged or confidential commercial or financial information.

VI–F–2. If NIH receives a request under the Freedom of Information Act for information so designated, NIH will promptly contact the institution to secure its views as to whether the information (or some portion) should be released.

VI–F–3. If the NIH decides to release this information (or some portion) in response to a Freedom of Information request or otherwise, the institution will

be advised; and the actual release will not be made until the expiration of 15 days after the institution is so advised, except to the extent that earlier release, in the judgement of the Director, NIH, is necessary to protect against an imminent hazard to the public or the environment.

VI–F–4. Projects should be registered in accordance with procedures specified in the *Administrative Practices Supplement.* The following information will usually be considered publicly available information, consistent with the need to protect proprietary data:

a. The names of the institution and principal investigator.

b. The location where the experiments will be performed.

c. The host-vector system.

d. The source of the DNA.

e. The level of physical containment.

VI–F–5–a. Any institution not otherwise covered by the Guidelines, which is considering submission of data or information voluntarily to NIH, may request presubmission review of the records involved to determine whether, if the records are submitted, NIH will or will not make part or all of the records available upon request under the Freedom of Information Act.

VI–F–5–b. A request for presubmission review should be submitted to ORDA, along with the records involved. These records must be clearly marked as being the property of the institution, on loan to NIH solely for the purpose of making a determination under the Freedom of Information Act. ORDA will then seek a determination from the HEW Freedom of Information Officer, the responsible official under HEW regulations (45 CFR Part 5), as to whether the records involved (or some portion) are or are not available to members of the public under the Freedom of Information Act. Pending such a determination, the records will be kept separate from ORDA files, will be considered records of the institution and not ORDA, and will not be received as part of ORDA files. No copies will be made of the records.

VI–F–5–c. ORDA will inform the institution of the HEW Freedom of Information Officer's determination and follow the institution's instructions as to whether some or all of the records involved are to be returned to the institution or to become a part of ORDA files. If the institution instructs ORDA to return the records, no copies or summaries of the records will be made or retained by HEW, NIH, or ORDA.

VI–F–5–d. The HEW Freedom of Information Officer's determination will represent that official's judgement, as of the time of the determination, as to whether the records involved (or some portion) would be exempt from disclosure under the Freedom of Information Act, if at the time of the determination the records were in ORDA files and a request was received from them under the Act.

Appendix A—Exemptions Under I–E–4

Section I–E–4 states that exempt from these Guidelines are "certain specified recombinant DNA molecules that consist entirely of DNA segments from different species that exchange DNA by known physiological processes, though one or more of the segments may be a synthetic equivalent. A list of such exchangers will be prepared and periodically revised by the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment (see Section IV–E–1–b–(1)–(d).) Certain classes are exempt as of publication of these Revised Guidelines. The list is in Appendix A."

Under exemption I–E–4 of these revised Guidelines are recombinant DNA molecules that are (1) composed entirely of DNA segments from one or more of the organisms within a sublist and (2) to be propagated in any of the organisms within a sublist. (Classification of *Bergey's Manual of Determinative Bacteriology,* eighth edition. R. E. Buchanan and N. E. Gibbons, editors. Williams and Wilkins Company: Baltimore, 1974.)

Sublist A

1. Genus *Escherichia*
2. Genus *Shigella*
3. Genus *Salmonella* (including *Arizona*)
4. Genus *Enterobacter*
5. Genus *Citrobacter* (including *Levinea*)
6. Genus *Klebsiella*
7. Genus *Erwinia*
8. *Pseudomonas aeruginosa, Pseudomonas putida* and *Pseudomonas fluorescens*
9. *Serratia marcescens*

Sublist B

1. *Bacillus subtilis*
2. *Bacillus licheniformis*
3. *Bacillus pumilus*
4. *Bacillus globigii*
5. *Bacillus niger*
6. *Bacillus nato*
7. *Bacillus amyloliquefaciens*
8. *Bacillus aterrimus*

Sublist C

1. *Streptomyces aureofaciens*
2. *Streptomyces rimosus*
3. *Streptomyces coelicolor*

Sublist D

1. *Streptomyces griseus*
2. *Streptomyces cyaneus*
3. *Streptomyces venezuelae*

Sublist E

One way transfer of *Streptococcus mutans* DNA into *Streptococcus sanguis.*

Sublist F

1. *Streptococcus sanguis*
2. *Streptococcus pneumoniae*

Appendix B—Classification of Micro-Organisms on the Basis of Hazard

I. Classification of Etiologic Agents on the Basis of Hazard (*1*)

A. Class 1 Agents

All bacterial, parasitic, fungal, viral, rickettsial, and chlamydial agents not included in higher classes.

B. Class 2 Agents

1. Bacterial Agents

Actinobacillus—all species except A. *mallei,* which is in Class 3
Arizona hinshawii—all serotypes
Bacillus anthracis
Bordetella—all species
Borrelia recurrentis, B. vincenti
Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani
Corynebacterium diptheriae, C. equi, C. haemolyticum, C. pseudotuberculosis C. pyogenes, C. renale
Diplococcus (Streptococcus) pneumoniae
Erysipelothrix insidiosa
Escherichia coli—all enteropathogenic serotypes
Haemophilus ducreyi, H. influenzae
Herellae vaginicola
Klebsiella—all species and all serotypes
Leptospira interrogans—all serotypes
Listeria—all species
Mima polymorpha
Moraxella—all species
Mycobacteria—all species except those listed in Class 3
Mycoplasma—all species except *Mycoplasma mycoides* and *Mycoplasma agalactiae,* which are in Class 5
Neisseria gonorrhoeae, N. meningitidis
Pasteurella—all species except those listed in Class 3
Salmonella—all species and all serotypes
Shigella—all species and all serotypes
Sphaerophorus necrophorus
Staphylococcus aureus
Streptobacillus moniliformis
Streptococcus pyogenes
Treponema carateum, T. pallidum, and T. pertenue
Vibrio fetus, V. comma, including biotype El Tor, and *V. parahemolyticus*

2. Fungal Agents

**Actinomycetes* (including *Nocardia* species and *Actinomyces species*

and Arachnia propionica)
Blastomyces dermatitidis
Cryptococcus neoformans
Paracoccidioides brasiliensis

3. Parasitic Agents
Endamoeba histolytica
Leishmania sp.
Naegleria gruberi
Toxoplasma gondii
Toxocara canis
Trichinella spiralis
Trypanosoma cruzi

4. Viral, Rickettsial, and Chlamydial Agents
Adenoviruses—human—all types
Cache Valley virus
Coxsackie A and B viruses
Cytomegaloviruses
Echoviruses—all types
Encephalomyocarditis virus (EMC)
Flanders virus
Hart Park virus
Hepatitis-associated antigen material
Herpes viruses—except *Herpesvirus simiae* (Monkey B virus) which is in Class 4
Corona viruses
Influenza viruses—all types except A/PR8/34, which is in Class 1
Langat virus
Lymphogranuloma venereum agent
Measles virus
Mumps virus
Parainfluenza virus—all types except Parainfluenza virus 3, SF4 strain, which is in Class 1
Polioviruses—all types, wild and attenuated
Poxviruses—all types except *Alastrim, Smallpox, Monkey pox, and Whitepox*, which depending on experiments, are in Class 3 or Class 4
Rabies virus—all strains except *Rabies street virus*, which should be classified in Class 3 when inoculated into carnivores
Reoviruses—all types
Respiratory syncytial virus
Rhinoviruses—all types
Rubella virus
Simian viruses—all types except *Herpesvirus simiae (Monkey B virus) and Marburg virus*, which are in Class 4
Sindbis virus
Tensaw virus
Turlock virus
Vaccinia virus
Varicella virus
Vole rickettsia
Yellow fever virus, 17D vaccine strain

C. Class 3 Agents
1. Bacterial Agents
Actinobacillus mallei *
Bartonella—all species
Brucella—all species
Francisella tularensis
Mycobacterium avium, M. bovis, M. tuberculosis
Pasteurella multocide type B ("buffalo" and other foreign virulent strains *)
Pseudomonas pseudomallei *
Yersenia pestis

2. Fungal Agents
Coccidioides immitis
Histoplasma capsulatum
Histoplasma capsulatum var. *duboisii*

3. Parasitic Agents
Schistosoma mansoni

4. Viral, Rickettsial, and Chlamydial Agents
***Alastrim, Smallpox, Monkey pox, and Whitepox*, when used *in vitro*
Arboviruses—all strains except those in Class 2 and 4 (Arboviruses indigenous to the United States are in Class 3, except those listed in Class 2. *West Nile* and *Semliki Forest* viruses may be classified up or down, depending on the conditions of use and geographical location of the laboratory.)
Dengue virus, when used for transmission or animal inoculation experiments
Lymphocytic choriomeningitis virus (LCM)
Psittacosis-Ornithosis-Trachoma group of agents
Rabies street virus, when used in inoculations of carnivores (See Class 2)
Rickettsia—all species except *Vole rickettsia* when used for transmission or animal inoculation experiments
Vesicular stomatitis virus *
Yellow fever virus—wild, when used *in vitro*

D. Class 4 Agents
1. Bacterial Agents: None
2. Fungal Agents: None
3. Parasitic Agents: None
4. Viral, Rickettsial, and Chlamydial Agents
***Alastrim, Smallpox, Monkey pox, and Whitepox*, when used for transmission or animal inoculation experiments
Hemorrhagic fever agents, including *Crimean hemorrhagic fever, (Congo), Junin*, and *Machupo* viruses, and others as yet undefined
Herpesvirus simiae (Monkey B virus)
Lassa virus
Marburg virus
Tick-borne encephalitis virus complex, including *Russian spring-summer encephalitis, Kyasanur forest disease, Omsk hemorrhagic fever*, and *Central European encephalitis viruses*
Venezuelan equine encephalitis virus, epidemic strains, when used for transmission or animal inoculation experiments
Yellow fever virus—wild, when used for transmission or animal inoculation experiments

II. Classification of Oncogenic Viruses on the Basis of Potential Hazard (*2*)

A. Low-Risk Oncogenic Viruses
Rous Sarcoma
SV–40
CELO
Ad7–SV40
Polyoma
Bovine papilloma
Rat mammary tumor
Avian Leukosis
Murine Leukemia
Murine Sarcoma
Mouse mammary tumor
Rat Leukemia
Hamster Leukemia
Bovine Leukemia
Dog Sarcoma
Mason-Pfizer Monkey Virus
Marek's
Guinea Pig Herpes
Lucke (Frog)
Adenovirus
Shope Fibroma
Shope Papilloma

B. Moderate-Risk Oncogenic Viruses
Ad2–SV40
FeLV
HV Saimiri
EBV
SSV–1
GaLV
HV ateles
Yaba
FeSV

III. Animal Pathogens (*3*)

A. Animal disease organisms which are forbidden entry into the United States by Law (CDC Class 5 agents)
1. Foot and mouth disease virus

B. Animal disease organisms and vectors which are forbidden entry into the United States by USDA Policy (CDC Class 5 Agents)
African horse sickness virus
African swine fever virus
Besnoitia besnoiti
Borna disease virus
Bovine infectious petechial fever
Camel pox virus
Ephemeral fever virus
Fowl plague virus
Goat pox virus
Hog cholera virus
Louping ill virus
Lumpy skin disease virus
Nairobi sheep disease virus
Newcastle disease virus (Asiatic strains)
Mycoplasma mycoides (contagious bovine pleuropneumonia)
Mycoplasma agalactiae (contagious

agalactia of sheep)
Rickettsia ruminatium (heart water)
Rift valley fever virus
Rhinderpest virus
Sheep pox virus
Swine vesicular disease virus
Teschen disease virus
Trypanosoma vivax (Nagana)
Trypanosoma evansi
Theileria parva (East Coast fever)
Theileria annulata
Theileria lawrencei
Theileria bovis
Theileria hirci
Vesicular exanthema virus
Wesselsbron disease virus
Zyonema

Footnotes and References of Appendix B

*A USDA permit, required for import and interstate commerce of pathogens, may be obtained from the Animal and Plant Health Inspection Service, USDA, Federal Building, Hyattsville, MD. 20782.

**Since the publication of the classification in 1974 (*1*), the *Actinomycetes* have been reclassified as bacterial rather than fungal agents.

***All activities, including storage of variola and whitepox, are restricted to the single national facility (World Health Organization (WHO) Collaborating Center for Smallpox Research, Center for Disease Control, in Atlanta).

1. *Classification of Etiologic Agents on the Basis of Hazard.* (4th Edition, July 1974). U.S. Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Office of Biosafety, Atlanta, Georgia 30333.
2. *National Cancer Institute Safety Standards for Research Involving Oncogenic Viruses* (October 1974). U.S. Department of Health, Education, and Welfare Publication No. (NIH) 75-790.
3. U.S. Department of Agriculture, Animal and Plant Health Inspection Service.

Appendix C—Exemptions Under I-E-5

Section I-E-5 states that exempt from these Guidelines are "Other classes of recombinant DNA molecules, if the Director, NIH, with advice of the Recombinant DNA Advisory Committee, after appropriate notice and opportunity for public comment, finds that they do not present a significant risk to health or the environment. (See Section IV-E-1-b-(1)-(d).) Certain classes are exempt as of publication of these Revised Guidelines."

Under exemption I-E-5 of these Revised Guidelines are those recombinant DNA molecules that are propagated and maintained in cells in tissue culture and that are derived entirely from non-viral components (that is, no component is derived from a eukaryotic virus).

Appendix D—HV1 and HV2 Host-Vector Systems Assigned Containment Levels as Specified in the Subsections of Section III-A

As noted above at the beginning of Section III-A, certain HV1 and HV2 host-vector systems are assigned containment levels as specified in the subsections of Section III-A. Those so classified as of publication of these Revised Guidelines are listed below.

* HV1—The following specified strains of *Neurospora crassa* which have been modified to prevent aerial dispersion:
(1) inl (inositolless) strains 37102, 37401, 46316, 64001 and 89601.
(2) csp-1 strain UCLA37 and csp-2 strains FS 590, UCLA101 (these are conidial separation mutants).
(3) eas strain UCLA191 (an "easily wettable" mutant).

HV1—Asporogenic mutant derivatives of *B. subtilis*. These derivatives must not revert to sporeformers with a frequency greater than 10^{-7}; data confirming this requirement must be presented to NIH for certification. The following plasmids are accepted as the vector components of certified *B. subtilis* HV1 systems: pUB110, pC194, pS194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124. *B. subtilis* strains RUB 331 and BGSC 1S53 have been certified as the host component of HV1 systems based on these plasmids.

HV2—The asporogenic mutant derivative of *Bacillus subtilis*, ASB298, with the following plasmids as the vector component: pUB110, pC194, pSA2100, pE194, pT127, pUB112, pC221, pC223, and pAB124.

Appendix E—Actions Taken Under the Guidelines

As noted in the subsections of Sections IV-E-l-b-(1) and IV-E-l-b-(2), the Director, NIH, may take certain actions with regard to the Guidelines after consideration by the RAC.

Some of the actions taken to date include the following:

1. The following experiment has been approved: The cloning in *B. subtilis*, under P2 conditions, of DNA derived from *Saccharomyces cerevisiae* using EK2 plasmid vectors provided that an HVl *B. subtilis* host is used.

2. Unmodified laboratory strains of *Neurospora crassa* can be used in all experiments for which HVl *N. crassa* systems are approved provided that these are carried out at physical containment one level higher than required for HVl. However, if P3 containment is specified for HVl *N. crassa*, this level is considered adequate for unmodified *N. crassa*. For P2 physical containment, special care must be exercised to prevent aerial dispersal of macroconidia, including the use of a biological safety cabinet.

3. P2 physical containment shall be used for DNA recombinants produced between members of the *Actinamycetes* group except for the species which are known to be pathogenic for man, animals, or plants.

4. Cloned desired fragments from any non-prohibited source may be transferred into *Agrobacterium tumefaciens* containing a Ti plasmid (or derivatives thereof), using a nonconjugative *E. coli* plasmid vector coupled to a fragment of the Ti plasmid and/or the origin of replication of an *Agrobacterium* plasmid, under containment conditions one step higher than would be required for the desired DNA in HVl systems (i.e. one step higher physical containment than that specified in the subsections of Section III-A). Transfer into plant parts or cells in culture would be permitted at the same containment level (one step higher).

5. *Bacillus subtilis* strains that do not carry an asporogenic mutation can be used as hosts specifically for the cloning of DNA derived from *E. coli* K-12 and *Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus,* and *S. venezuelae,* using NIH-approved *Staphylococcus aureus* plasmids as vectors under P2 conditions.

6. *Streptomyces coelicolor, S. aureofaciens, S. rimosus, S. griseus, S. cyaneus, and S. venezuelae* can be used as hosts for the cloning of DNA derived from *B. subtilis, E. coli* K-12 or from *S. aureus* vectors that have been approved for use in *B. subtilis* under P2 conditions, using as vectors any plasmid indigenous to *Streptomyces* species or able to replicate in these hosts by natural biological mechanisms.

7. Certain cloned segments of *Anabena* DNA may be transferred into *Klebsiella* under P2 physical containment.

8. Permission is granted to clone foot-and-mouth disease virus in the EKlCV host-vector system consisting of *E. coli* K-12 and the vector pBR322, all work to be done at the Plum Island Animal Disease Center.

9. Permission is granted to clone the Exotoxin A gene of *Pseudomonas aeruginosa* under P1 + EK1 conditions

* These follow the assigned containment levels as specified in the subsections of Section III-A with one exception. This exception is that experiments involving complete genomes of eukaryotic viruses will require P3 + HV1 or P2 + HV2 rather than the levels given in the subsections of Section III-A.

in *Escherichia coli* K–12 and under P1 conditions in *Pseudomonas aeruginosa*.

10. Permission is granted to return to the host of origin *Helminthosporanium maydis* (race O) DNA which has been cloned in yeast strain SHY2 using the hybrid *E. coli*—yeast plasmid Y1p5. The cloned DNA may be returned to, and propagated in, *Helminthosporanium maydis* at the P2 level of physical containment.

11. Permission is granted to return *Schizophyllum commune* DNA (or yeast DNA) cloned in *Saccharomyces cerevisiae* with YR or 2 mu circle vectors to *Schizophyllum commune*. The cloned DNA may be returned to, and propagated in, *Schizophyllum commune* at the P2 level of physical containment.

12. Permission is granted to return *Wangiella dermatitidis* DNA to *Wangiella dermatitidis* using an HV2 certified *Saccharomyces/E. coli* hybrid vector. The *Wangiella dermatitidis* may be propagated at the P3 level of physical containment.

13. Certain specified clones derived from segments of the Foot-and-Mouth Disease Virus may be transferred from Plum Island Animal Disease Center to the facilities of Genentech, Inc., of South San Fransico, California. Further development of the clones at Genentech has been approved under P1 + EK1 conditions.

14. *Saccharomycopsis lipolytica* may be used as a host for tranformation with defined *Escherichia coli/Saccharomyces cerevisiae* hybrid plasmids and the hybrid plasmids may be used for cloning *S. lipolytica* DNA in *E. coli* and returning the cloned DNA to *S. lipolytica*.

15. Conjugative plasmids or transducing phages may be employed in recombinant DNA experiments when employing *E. coli* as host when a small defined segment of Adenovirus 2 DNA is employed as linker DNA.

16. Permission is granted to introduce DNA segments from aphid transmissible strains into non-aphid transmissible strains of Cauliflower mosaic virus in order to study the factors determining aphid transmissibility.

17. Permission is granted to return *Mucor racemosus* DNA which has been cloned in *Saccharomyces cerevisiae* host-vector systems to *Mucor racemosus*. In addition, permission is granted to transform *Mucor racemosus* with *S. cerevisiae* vectors with or without cloned *S. cerevisiae sequences*. These manipulations may be performed under P2 conditions.

18. *Schizosaccharomyces pombe* DNA may be cloned in *Schizosaccharomyces pombe* using approved HV1 *Saccharomyces cerevisiae/E. coli* hybrid plasmids as vectors under P1 containment conditions.

19. The pyrogenic endotoxin type A (Tox A) gene of *Staphylococcus aureus* may be cloned in an HV2 *Bacillus subtilis* host-vector system under P3 containment conditions.

20. A hybrid plasmid composed of, (1) *E. coli* plasmid pBR325, (2) the origin of replication and transfer genes of *Agrobacterium tumefaciens* plasmid Ti, (3) the thiamine gene of *E. coli*, and (4) *Arabidopsis* DNA, may be transformed into *Agrobacterium tumefaciens* under P1 conditions. The *Agrobacterium tumefaciens* may subsequently be used to introduce the composite plasmid carrying *Arabidopsis* DNA and the *E. coli* thiamine gene into *Arabidopsis* plants under P1 containment conditions.

21. *Chlamydomonas reinhardi* can be used as a host for cloning defined DNA segments derived from *E. coli* and *Saccharomyces cerevisiae* using *E. coli/S. cerevisiae* hybrid vectors under P2 physical containment.

22. *Candida albicans* can be used as a host for cloning *Candida albicans* DNA following propagation of the DNA in *E. coli* K–12 or in *Saccharomyces cerevisiae* employing an *E. coli-S. cerevisiae* hybrid plasmid vector or the yeast 2 micron plasmid.

23. The Rd strain of *Hemophilus influenzae* can be used as a host for the propagation of the cloned Tn 10 tet R gene derived from *E. coli* K–12 employing the non-conjugative *Haemophilus* plasmid, pRSF0885, under P1 conditions.

24. *Zymomonas mobilis* may be used as a host under P2 conditions for transformation by recombinant DNA derived from *Pseudomonas* strains that are non-pathogenic for animals or plants, and that has been cloned in an *E. coli* K–12 host.

25. Protoplasts of *Streptosporangium brasiliense* may be transformed with a hybrid plasmid containing pBR322 plus a *Streptosporangium* plasmid into which have been incorporated specified DNA segments from *Streptomyces* species or an HV1 approved *Bacillus subtilis* cloning vector.

Appendix F—Certified HV2 Host-Vector Systems

While the Guidelines no longer specify the use of *E. coli* K–12 EK2 or *Saccharomyces cerevisiae* HV2 systems, investigators may wish to employ these systems in specific instances. The currently certified EK2 and HV2 systems are:

HV2—The following sterile strains of *Saccharomyces cerevisiae*, all of which have the ste–VC9 mutation, SHY1, SHY2, SHY3, and SHY4. The following plasmids are certified for use: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, and YIp33.

EK2 Plasmid Systems. The *E. coli* K–12 strain chi–1776. The following plasmids are certified for use: pSC101, pMB9, pBR313, pBR322, pDH24, pBR327. The following *E. coli/S. cerevisiae* hybrid plasmids are certified as EK2 vectors when used in *E. coli* chi–1776 or in the sterile yeast strains, SHY1, SHY2, SHY3 and SHY4: YIp1, YEp2, YEp4, YIp5, YEp6, YRp7, YEp20, YEp21, YEp24, YIp25, YIp26, YIp27, YIp28, YIp29, YIp30, YIp31, YIp32, YIp33.

EK2 Bacteriophage Systems. The following are certified EK2 systems based on bacteriophage lambda:

Vector	*Host*
–gt*WES.–B*	DP50*supF*
–gt*WES.–B**	DP50*supF*
–gtZJvir.–*B*	*E. coli* K–12
–gtALO.–B	DP50*supF*
Charon 3A	DP50 or DP50*supF*
Charon 4A	DP50 or DP50*supF*
Charon 16A	DP50 or DP50*supF*
Charon 21A	DP50*supF*
Charon 23A	DP50 or DP50*supF*
Charon 24A	DP50 or DP50*supF*

Dated: November 14, 1980.

Donald S. Fredrickson,

Director, National Institutes of Health.

OMB's "Mandatory Information Requirements for Federal Assistance Program Announcements" (45 FR 39592) requires a statement concerning the official government program contained in the *Catalog of Federal Domestic Assistance*. Normally NIH lists in its annoucements the number and title of affected individual programs for the guidance

of the public. Because the guidance in this notice covers not only virtually every NIH program but also essentially every federal research program in which DNA recombinant molecule techniques could be used, it has been determined to be not cost effective or in the public interest to attempt to list these programs. Such a list would likely require several additional pages. In addition, NIH could not be certain that every federal program would be included as many federal agencies, as well as private organizations, both national and international, have elected to follow the NIH Guidelines. In lieu of the individual program listing, NIH invites readers to direct questions to the information address above about whether individual programs listed in the *Catalog of Federal Domestic Assistance* are affected.

NIH programs are not covered by OMB Circular A–95 because they fit the description of "program not considered appropriate" in Section 8–(b)–(4) and (5) of that Circular.

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